LECTURE NOTE

On

EMBRYOLOGY

VBA 203: DEVELOPMENTAL ANATOMY

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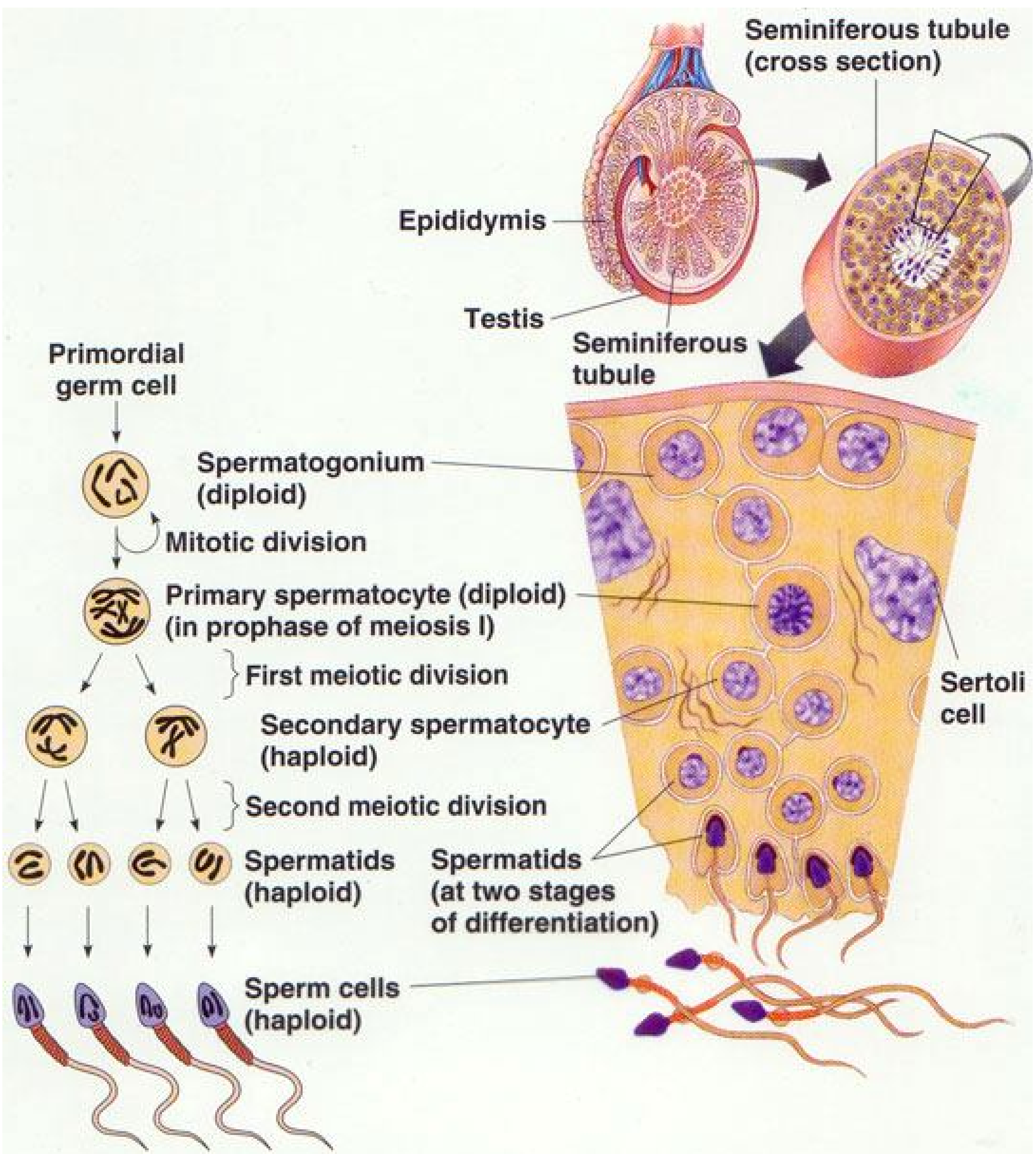
VBA 203: Embryology Lecture

- Gametogenesis : This is the formation of the gametes in both the male and female reproductive systems.
    - It is the major function of the gonads. In the male it is called spermatogenesis and in the female it is called the oogenesis.

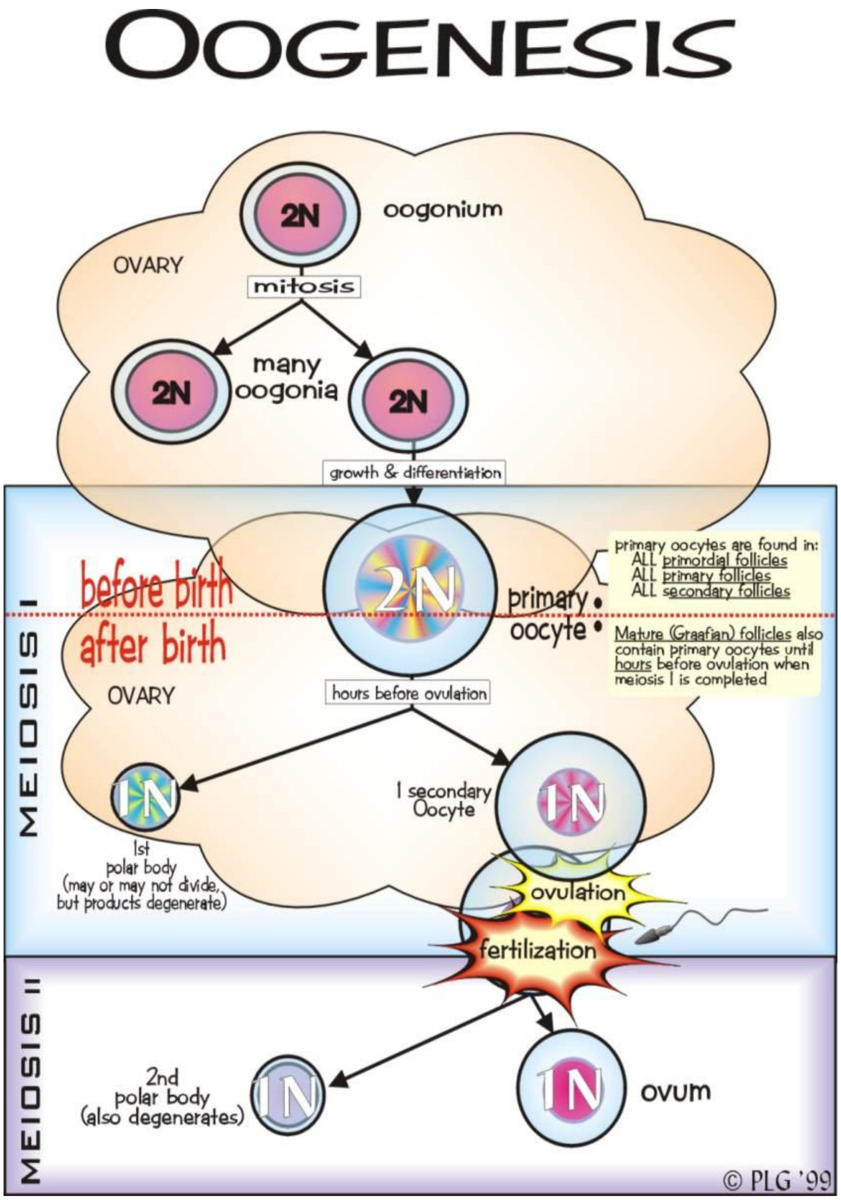
- Spermatogenesis begins when the cells of the germinal epithelium become the primordial germ cells as schematically represented below:

Fig. 1



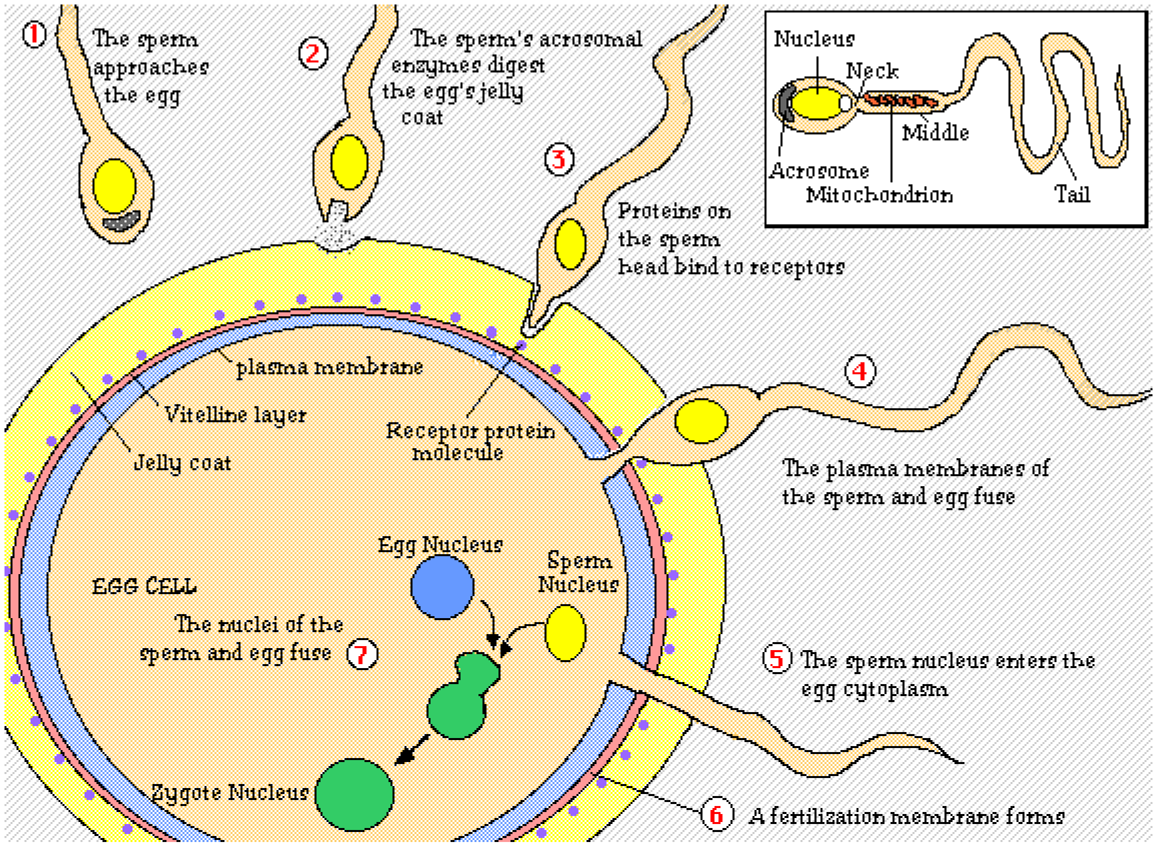
- In the same way the process of oogenesis begins when the cells germinal epithelium become the oogonia as is schematically depleted below:

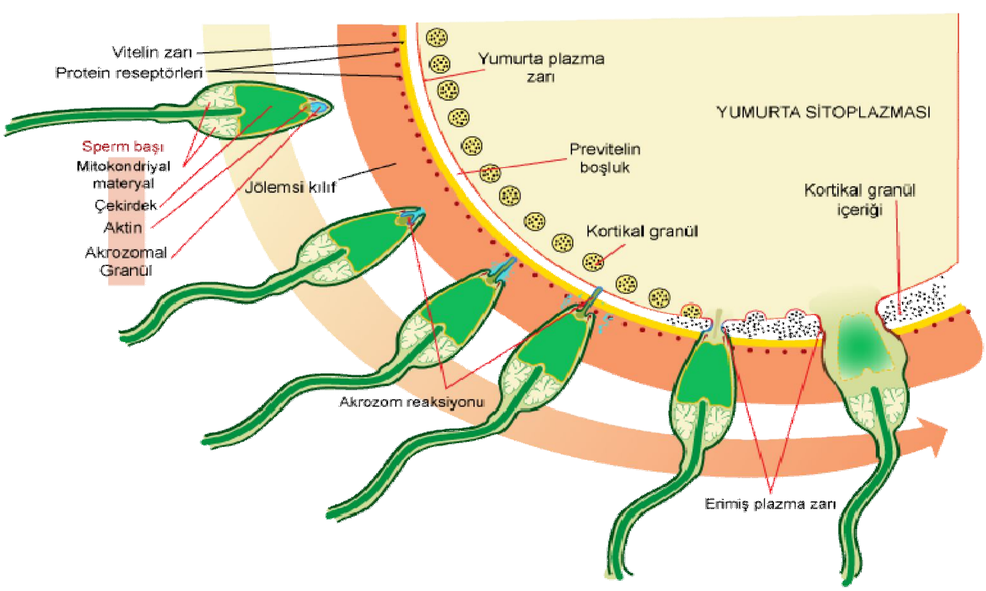
Fig. 2



* **Fertilization**: This is the fusion of the male and female gamete to form zygote.

Fig. 3





Fertilization is the union of two haploid gametes to reconstitute a diploid cell - a cell with the potential to become a new individual. A more detailed understanding of this process shows the it involve several steps that are outlined below:

In overview, fertilization can be described as the following steps:

Sperm Capacitation

- Freshly ejaculated sperm are unable or poorly able to fertilize.
- They must first undergo a series of changes known collectively as capacitation.
- Capacitation is associated with:
    - Removal of adherent seminal plasma proteins.
    - Reorganization of plasma membrane lipids and proteins.
    - It also seems to involve an influx of extracellular calcium, increase in cyclic AMP, and decrease in intracellular pH.
    - The molecular details of capacitation appear to vary somewhat among species.
- Capacitation occurs while sperm reside in the female reproductive tract for a period of time, as they normally do during gamete transport. The length of time required varies with species, but usually requires several hours.
- The sperm of many mammals, including humans, can also be capacitated by incubation in certain fertilization media.
- Sperm capacitation leads to hyper-activation (among other things) which is displayed by hyper-activated motility.
- Most importantly however, capacitation appears to destabilize the sperm's membrane in preparation for the acrosome reaction.

Sperm-Zona Pellucida Binding

- Binding of spermatozoon to the zona pellucida is a receptor-ligand interaction with a high degree of species specificity.
- The carbohydrate groups on the zona pellucida glycoproteins function as sperm receptors.
- The sperm molecule that binds this receptor is not known with certainty, and indeed, there may be several proteins that can serve this function.

The Acrosome Reaction

- After binding to the zona pellucida, the sperm then faces the herculean task of penetrating the zona pellucida to get to the oocyte.
- Evolution's response to this challenge is the acrosome - a huge modified lysosome that is packed with zona-digesting enzymes and located around the anterior part of the sperm's head.
- The acrosome reaction provides the sperm with an enzymatic drill to get throught the zona pellucida.
- The same zona pellucida protein that serves as a sperm receptor also stimulates a series of events that lead to many areas of fusion between the plasma membrane and outer acrosomal membrane.

- Membrane fusion (actually an exocytosis) and vesiculation expose the acrosomal contents, leading to leakage of acrosomal enzymes from the sperm's head.
- As the acrosome reaction progresses and the sperm passes through the zona pellucida, more and more of the plasma membrane and acrosomal contents are lost.
- By the time the sperm traverses the zona pellucida, the entire anterior surface of its head, down to the inner acrosomal membrane, is denuded.
- Sperm that lose their acrosomes before encountering the oocyte are unable to bind to the zona pellucida and thereby unable to fertilize.
- Assessment of acrosomal integrity of ejaculated sperm is commonly used in semen analysis.
- The combination of the constant propulsive force from the sperm's flagellating tail and acrosomal enzymatic activity allow the sperm to create a tract through the zona pellucida.
- These two factors - motility and zona-digesting enzymes- allow the sperm to traverse the zona pellucida.

Sperm-Oocyte Binding

- Once a sperm penetrates the zona pellucida, it binds to and fuses with the plasma membrane of the oocyte.
- Binding occurs at the posterior (post-acrosomal) region of the sperm head.
- The molecular nature of sperm-oocyte binding is not completely resolved. A leading candidate in some species is a dimeric sperm glycoprotein called **fertilin**, which binds to a protein in the oocyte plasma membrane and may also induce fusion.
- Interestingly, humans and apes have inactivating mutations in the gene encoding one of the subunits of fertilin, suggesting that they use a different molecule to bind oocytes.

Egg Activation and the Cortical Reaction

- Prior to fertilization, the egg is in a quiescent state, arrested in metaphase of the second meiotic division.
- Upon binding of a sperm, the egg rapidly undergoes a number of metabolic and physical changes that collectively are called *egg activation*.
- Prominent effects include a rise in the intracellular concentration of calcium, completion of the second meiotic division and the so-called cortical reaction.
- The cortical reaction refers to a massive exocytosis of cortical granules seen shortly after sperm-oocyte fusion.
- Cortical granules contain a mixture of enzymes, including several proteases, which diffuse into the zona pellucida following exocytosis from the egg.
- These proteases alter the structure of the zona pellucida, inducing what is known as the zona reaction.
- Components of cortical granules may also interact with the oocyte plasma membrane.

The Zona Reaction

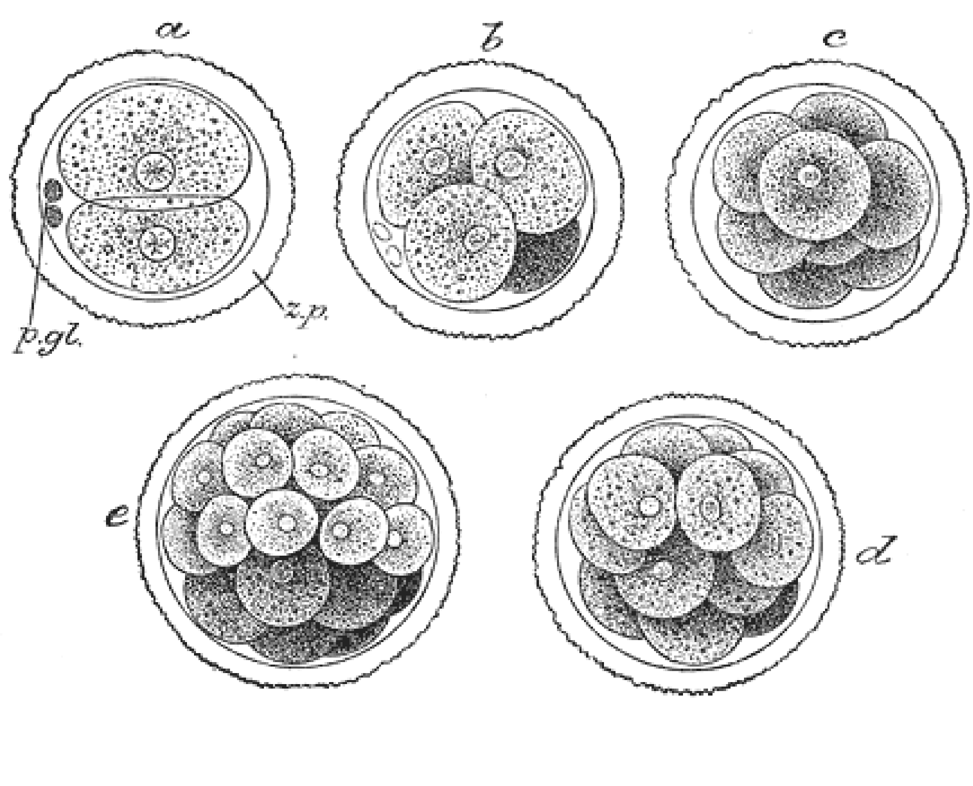
- The zona reaction refers to an alteration in the structure of the zona pellucida catalyzed by proteases from cortical granules. *The critical importance of the zona reaction is that it represents the major block to polyspermy* in most mammals.
- This effect is the result of two measurable changes induced in the zona pellucida:

1. *The zona pellucida hardens*. Crudely put, this is analogous to the setting of concrete. Runner-up sperm that have not finished traversing the zona pellucida by the time the hardening occurs are stopped in their tracks.
2. *Sperm receptors in the zona pellucida are destroyed*. Therefore, any sperm that have not yet bound to the zona pellucida will no longer be able to bind, let alone fertilize the egg.

The loss of sperm receptors can be demonstrated by mixing sperm with both unfertilized oocytes (which have not yet undergone the zona reaction) and two-cell embryos (which have previously undergone cortical and zona reactions). In this experiment, sperm attach avidly to the zona pellucida of oocytes, but fail to bind to the two-cell embryos.

Cleavage and Blastocyst formation:

Fig. 4



- Soon after the development of the 8-cell or 16-cell embryo (c - depending on the species), the blastomeres begins to form tight junctions with one another.
- This leads to deformation of their round shape and consequently forms a mulberry-shaped mass of cells called a **morula**.
- This change in shape of the embryo is called **compaction** - mediated by cell surface adhesion glycoproteins.
- It is difficult to count the cells in a morulla.
- After reaching the 16-cell stage, the cells of the morula differentiate.
- The inner blastomeres will become the inner cell mass and the blastomeres on the surface will later flatten to form the trophoblast.
- Formation of junctional complexes between blastomeres gives the embryo an outside and an inside.
- The outer cells of the embryo also begin to express a variety of membrane transport molecules, including sodium pumps.
- One result of these changes is an accumulation of fluid inside the embryo, which signals formation of the **blastocyst or blastula or blastodermic vesicle**. The fluid is called blastocoele.
- A blastocyst is composed of a hollow sphere of **trophoblast cells**, inside of which is a small cluster of cells called the **inner cell mass**. Trophoblast goes on to contribute to fetal membrane systems, while the inner cell mass is destined largely to become the embryo and fetus.

Gastrulation:

- After the formation of the inner cell mass, it undergoes differentiation to form two layers namely the epiblast and the hypoblast (fig5)
  Fig. 5

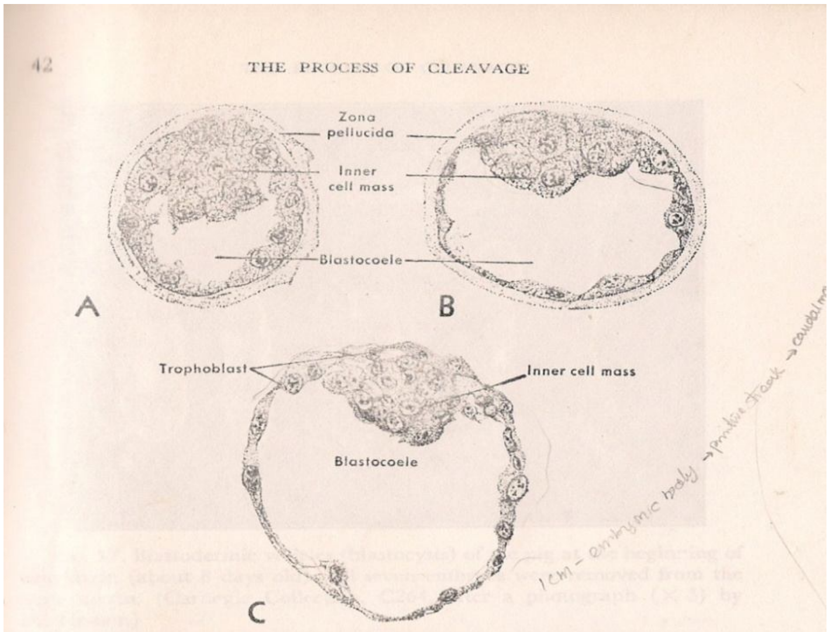
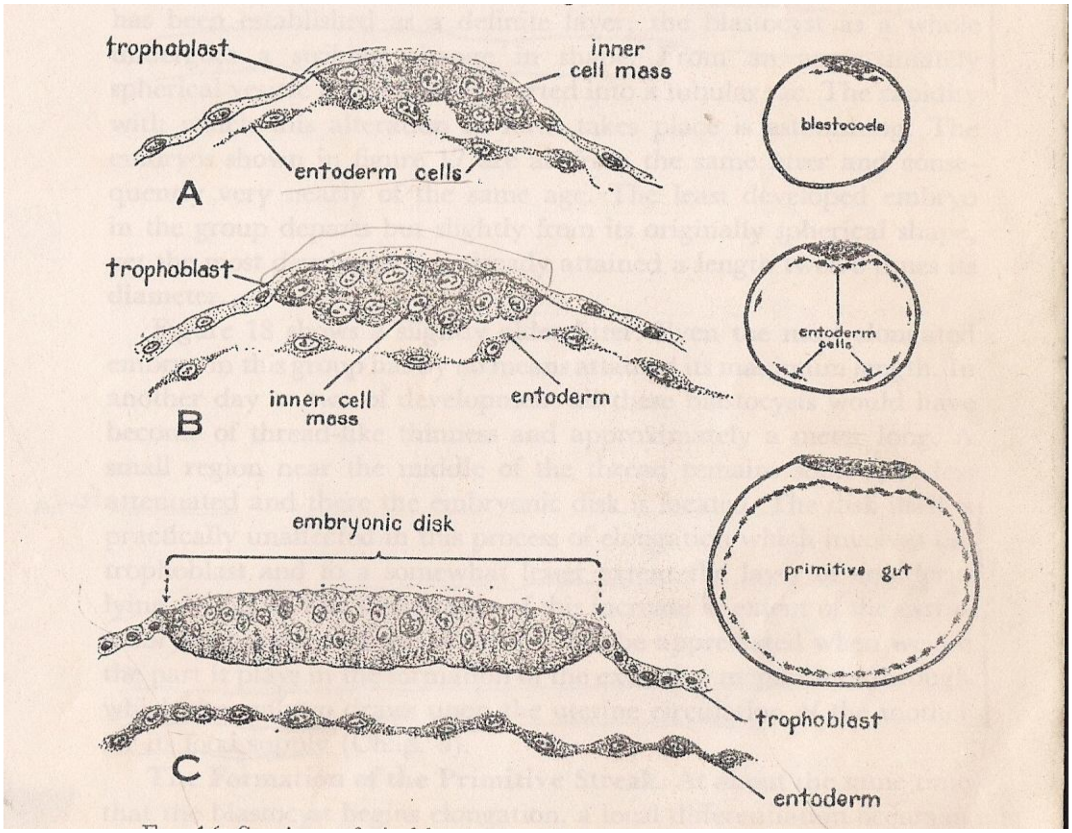


Fig. 6



- The trophoblast on the inner cell mass disappears and the mass becomes the embryonic disk.
- Gastrulation is the process of establishing the three germ layers of the embryo.
- The process starts with the formation of Primitive streak o the surface of the epiblast.
- The primitive streak is a narrow groove with slightly bulging regions on either side. It is made up of primitive node and pit.
- The cells of the epiblast migrate towards the primitive streak, and on arrival they become flask shaped, detach from the epiblast and slip beneath it through the process of invagination.
- The epiblast becomes the ectoderm, the invaginated cells become the mesoderm and hypoblast becomes the endoderm. The entire embryo becomes a gastrula.
- The formation of the gastrula marks the beginning of the process of organogenesis.

Fig. 7

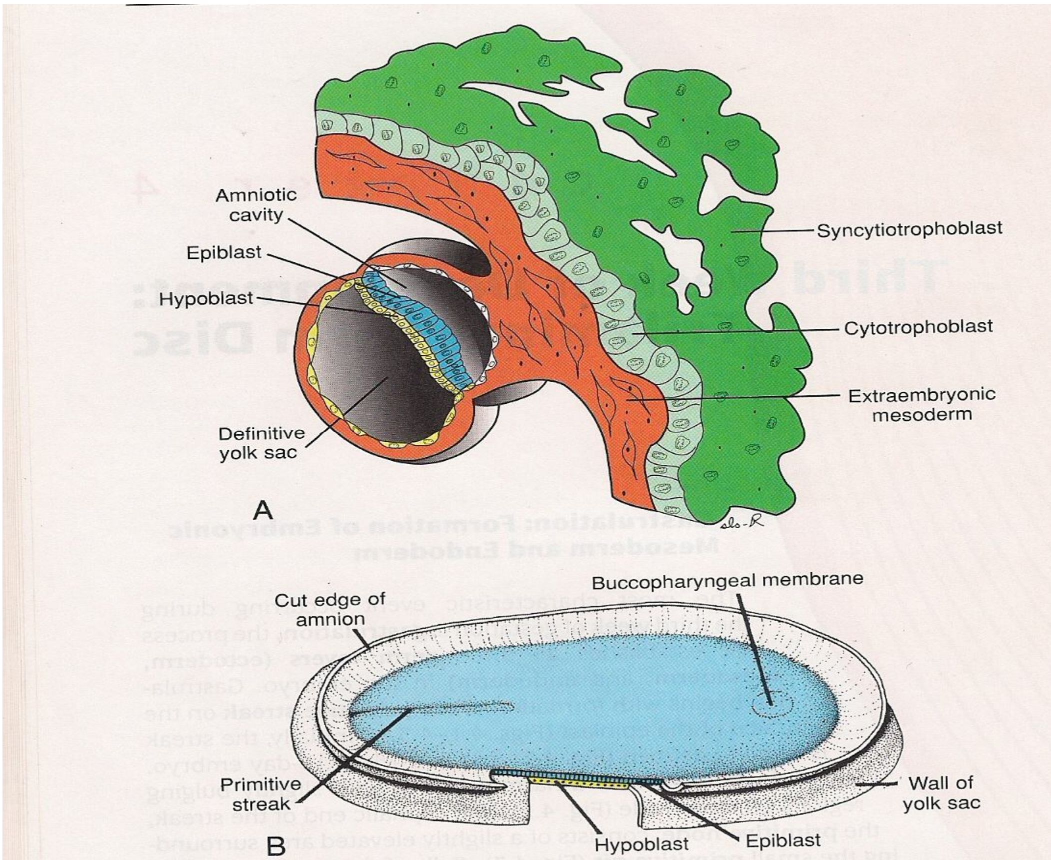

Amniotic cavity
Epiblast
Hypoblast
Definitive yolk sac
A
Syncytiotrophoblast
Cytotrophoblast
Extraembryonic mesoderm
Cut edge of amnion
Buccopharyngeal membrane
Primitive streak
B
Hypoblast
Epiblast
Wall of yolk sac

Fig. 8

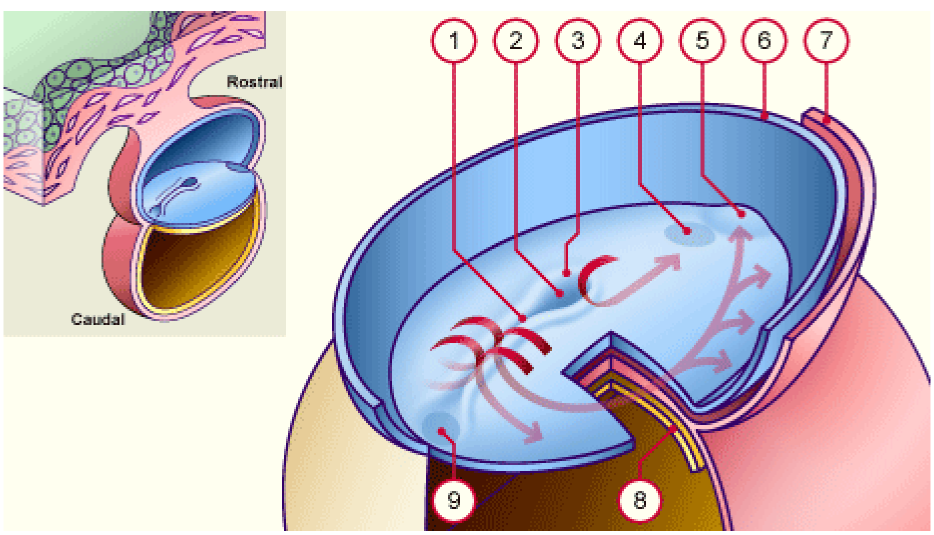
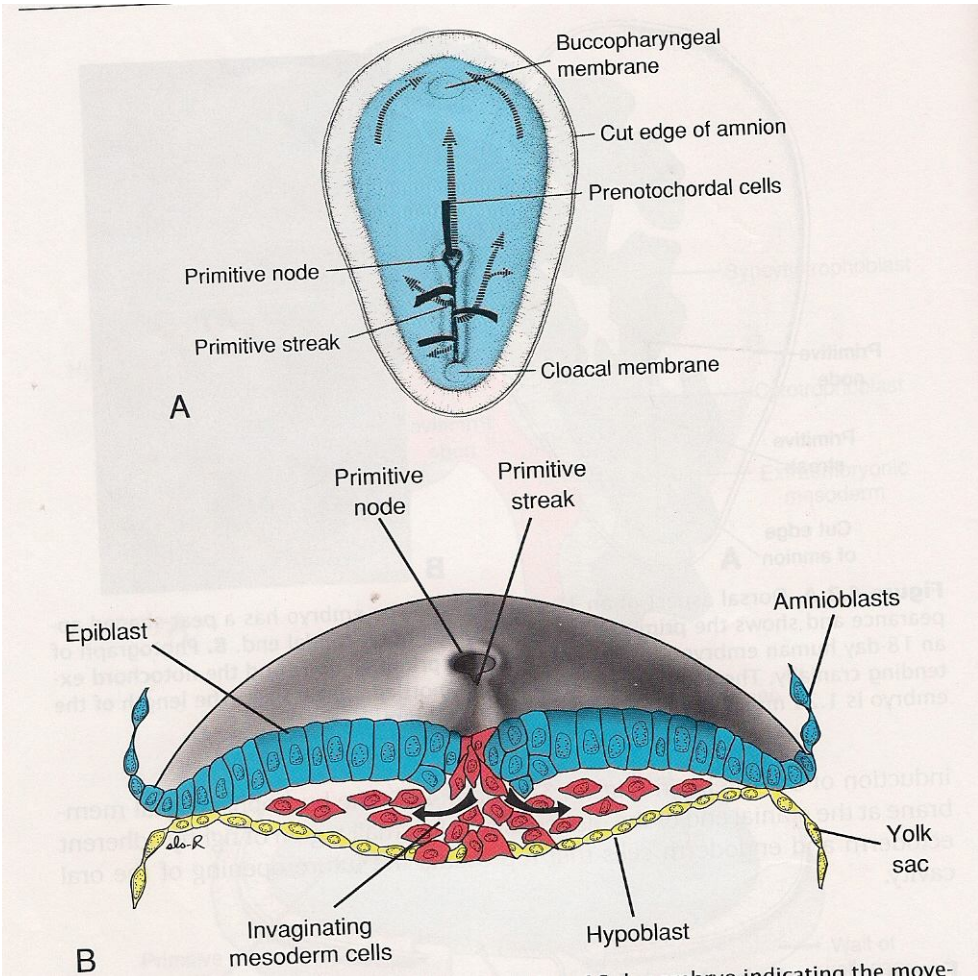

Rostral
Caudal
1
2
3
4
5
6
7
8
9

Fig. 9

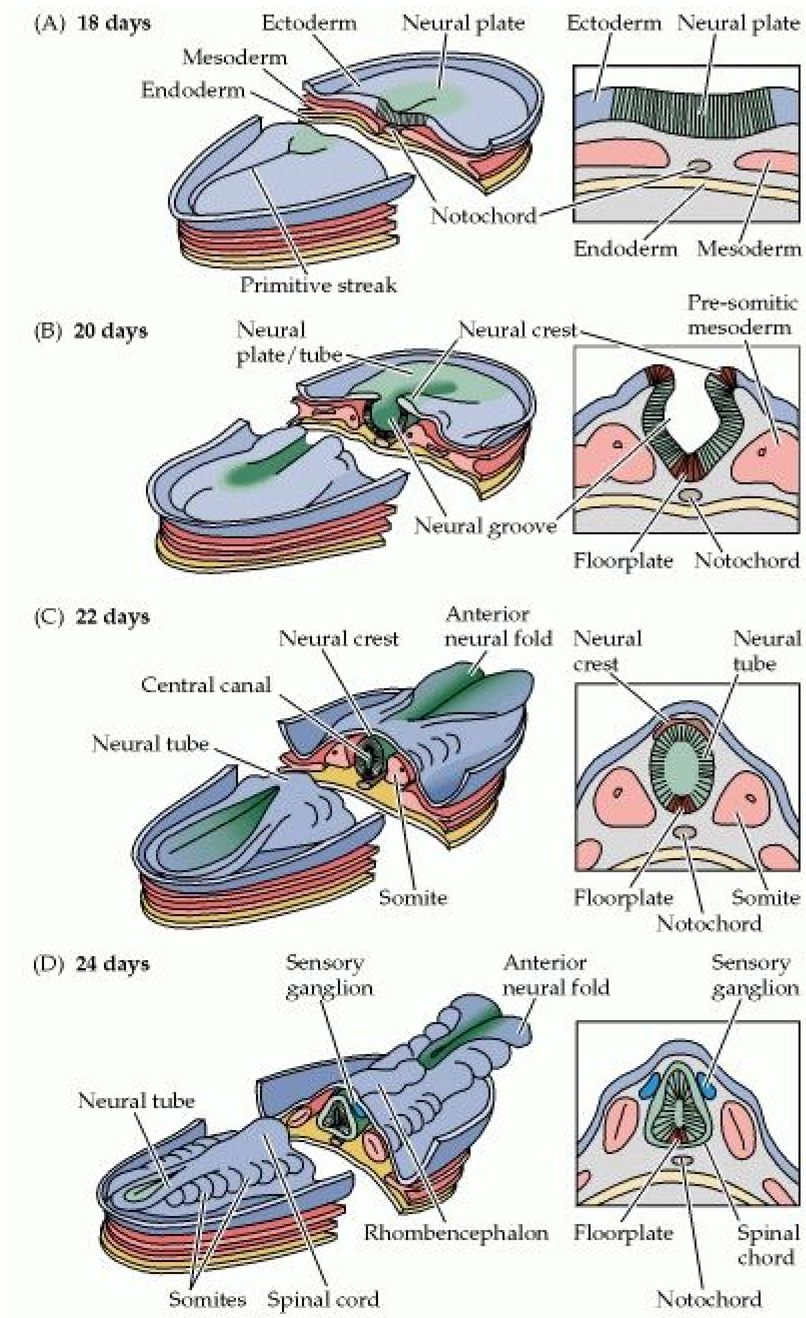


Formation of Notochord and body forms:

- Notochord is a solid cord of cells that is formed under the endoderm. It underlies the neural tube and serves as the basis for axial skeleton.
- As the embryo develops, the primitive streak marks the long axis and the caudal end of the body.
- With the formation of the Notochord, the embryo begins the formation of the Nervous system.

Formation of Nervous System:

Fig. 10



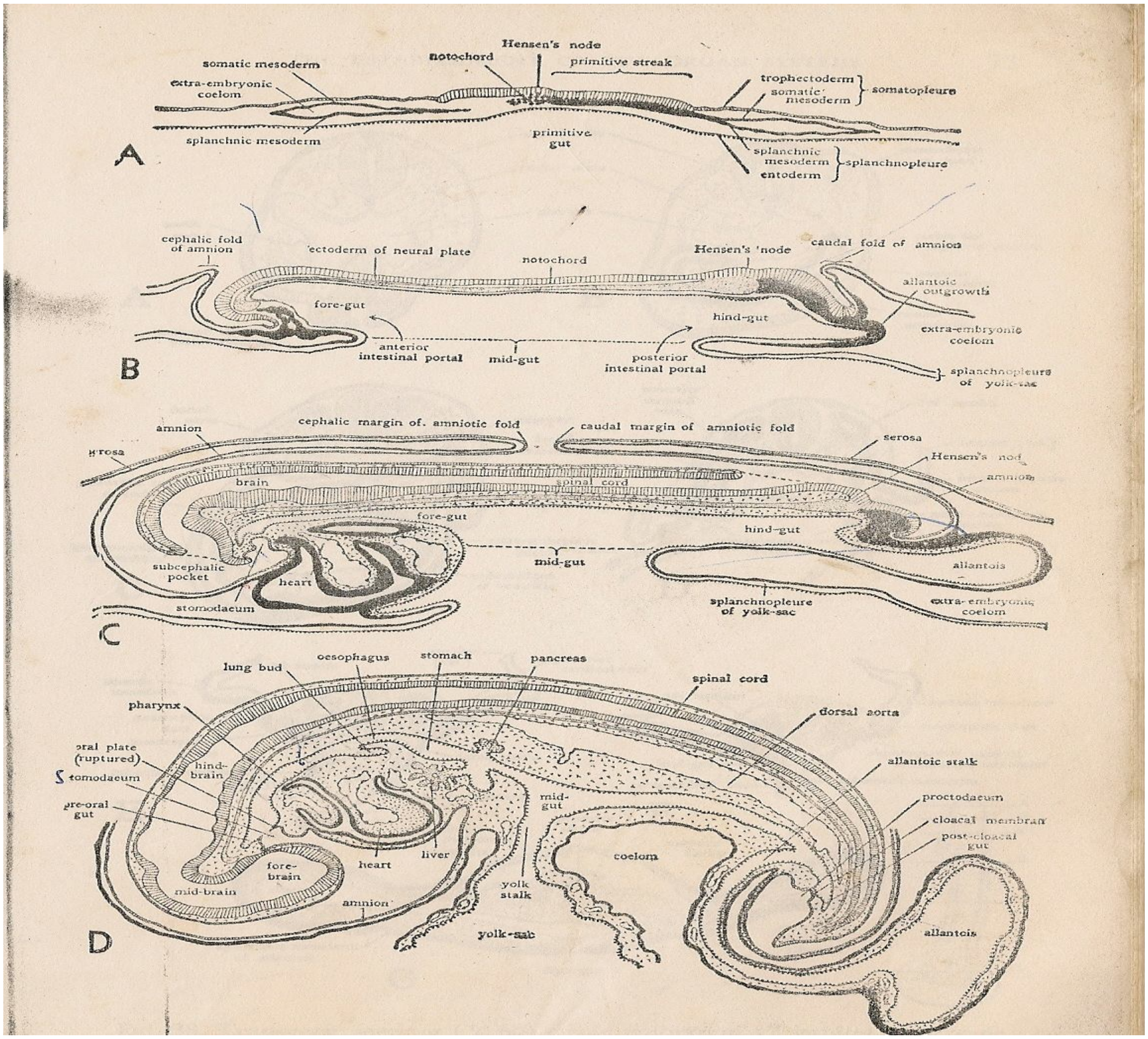
Neurulation in the mammalian embryo.

- On the left are dorsal views of the embryo at several different stages of early development; each boxed view on the right is a midline cross section through the embryo at the same stage.
- (A) During late gastrulation and early neurulation, the notochord forms by invagination of the mesoderm in the region of the primitive streak.
- (B) As neurulation proceeds, the neural plate begins to fold on itself, forming the neural groove and ultimately the neural tube. The neural plate immediately above the notochord differentiates into the floorplate, whereas the neural crest emerges at the lateral margins of the neural plate (farthest from the notochord).
- (C) Once the edges of the neural plate meet in the midline, the neural tube is complete. The mesoderm adjacent to the tube then thickens and subdivides into structures called somites—the precursors of the axial musculature and skeleton.
- (D) As development continues, the neural tube adjacent to the somites becomes the rudimentary spinal cord, and the neural crest gives rise to sensory and autonomic ganglia (the major elements of the peripheral nervous system). Finally, the anterior ends of the neural plate (anterior neural folds) grow together at the midline and continue to expand, eventually giving rise to the brain.
- The development of the brain starts with the development of three vesicles at the cephalic region. They are the prosencephalon (forebrain), the mesencephalon (midbrain) and the rhombencephalon.
- The Proencephalon divides to form the telencephalon and diencephalon.
- The mesencephalons remain undivided.
- The rhombencephalons divide to form the metencephalon and myelencephalon.

Formation of Digestive System:

The development of the digestive system starts early in the embryonic life as illustrated below.

Fig. 11



- Most of the <u>digestive system</u> (except for the majority of the oral cavity and anal canal, and some of the salivary glands) develops from the primitive gut, which was created by the folding of the trilaminar embryonic disc to form the embryo proper during the 4th week.
- Formation of the primitive gut yields one of the principal features of the vertebrate body plan - a tube within a tube.

The formation of the primitive gut

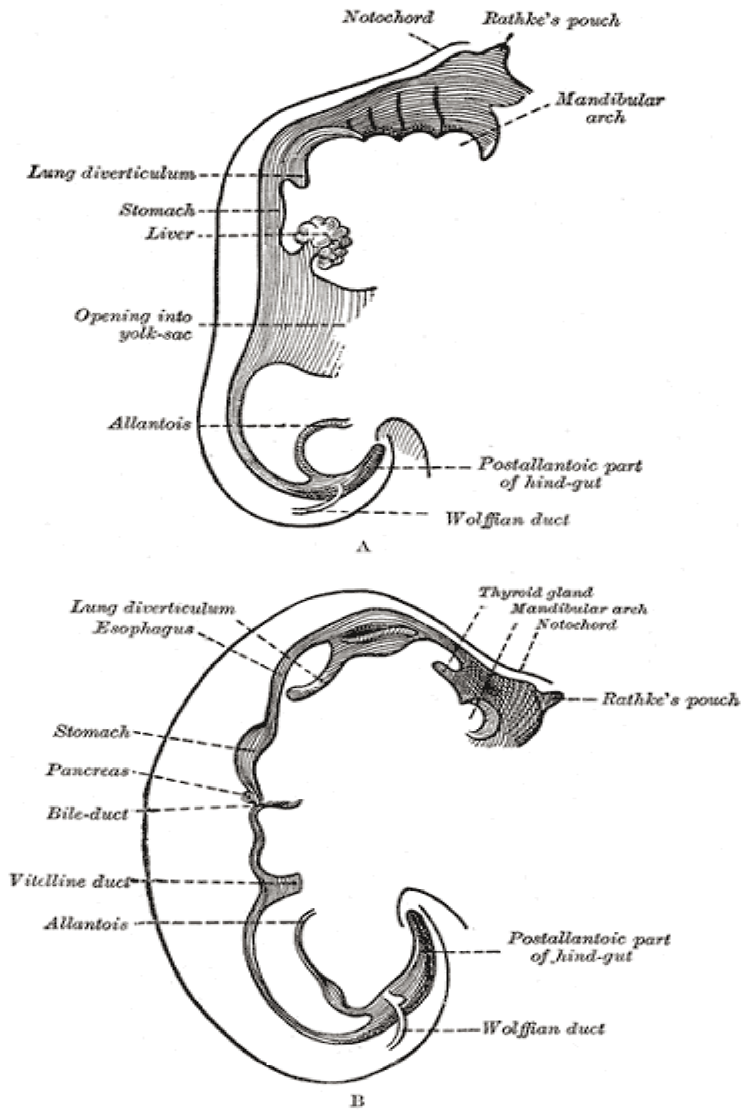
- The mucosal epithelium and associated glands of the gastrointestinal tract develop from endoderm. Embryonic endoderm is formed in the 3rd week as the ventral layer of the trilaminar embryonic disc.

- The connective tissue, muscle and mesothelium are derived from splanchnic mesoderm. The lateral plate region of mesoderm can be distinguished in the fourth week.
- In the posterior (caudal) half of the embryo, the lateral plate mesoderm splits to form a dorsal somatic mesoderm and a ventral splanchnic mesoderm.
- Somatic mesoderm with the overlying ectoderm is called the somatopleure; and splanchnic mesoderm and endoderm, the splanchnopleure.
- The enteric nervous system develops from neural crest cells.

Embryo formation by folding of the trilaminar embryo in the 4th week creates the primitive gut.

- Lateral folding of the splanchnopleure results in a midline tube along an anterior-posterior axis, and lateral folding of the somatopleure closes the body wall creating the periotoneum from trapped intra- and extra-embryonic coelom.
- Simultaneously, the connection between the primitive gut and the dorsal body wall thins to form a dorsal mesentery (and dorsal mesogastrium).
- Cranial folding of the embryo brings the anterior end of the embryonic disk down to the level of the distal end of the foregut, where it forms the septum transversum, and the prechordal plate to the cranial end of the primitive gut, where it forms the oropharyngeal (or buccopharyngeal) membrane. The septum transversum becomes the central tendon of the diaphragm and the ventral mesogastrium. After it is invaded by the liver bud, the ventral mesogastrium gives rise to the falciform ligament, capsule of the liver, and lesser omentum.
- Caudal folding of the embryo places a cloacal membrane at the terminal end of the primitive gut.
- The primitive gut is divided into three regions (foregut, midgut and hindgut).

Fig. 12



Development of the Foregut (weeks 4-7)

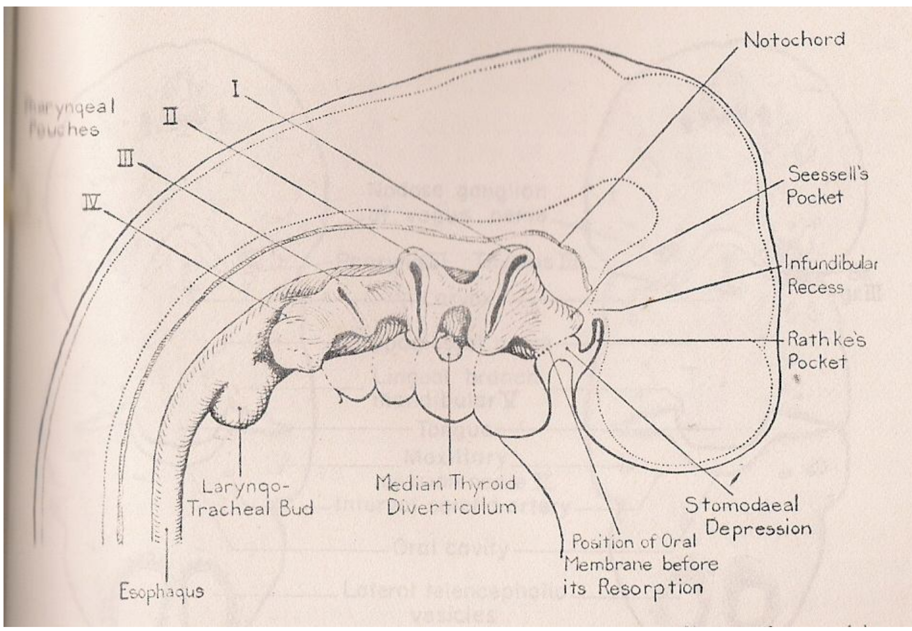
- The foregut extends from the oropharyngeal membrane to (and includes) the hepatocystic diverticulum (liver bud).
- The foregut can be further divided into cranial and caudal regions.

Cranial foregut:

- The cranial foregut, or pharyngeal gut, extends from the oropharyngeal membrane to (and includes) the respiratory diverticulum (lung bud).

- The derivatives of the pharyngeal gut include part of mouth and tongue, pharynx, thyroid, parathyroid, thymus, lower respiratory tract, and lungs.
- The pharyngeal gut and stomodeum both contribute to development of the mouth.
- The stomodeum is an ectodermal depression in the developing face that forms the primordial mouth.
- After the oropharyngeal membrane, which separates the stomodeum from the pharyngeal gut, ruptures in the 5th week, the stomodeum connects the amniotic cavity and the pharyngeal gut.
- Pharyngeal ("branchial") arches appear during the 4th and 5th weeks on the ventral side of the pharyngeal gut. Each arch has cartilage, a cranial nerve, an aortic arch artery and muscle.
- Pharyngeal clefts and pouches are located between the arches.

Fig. 13



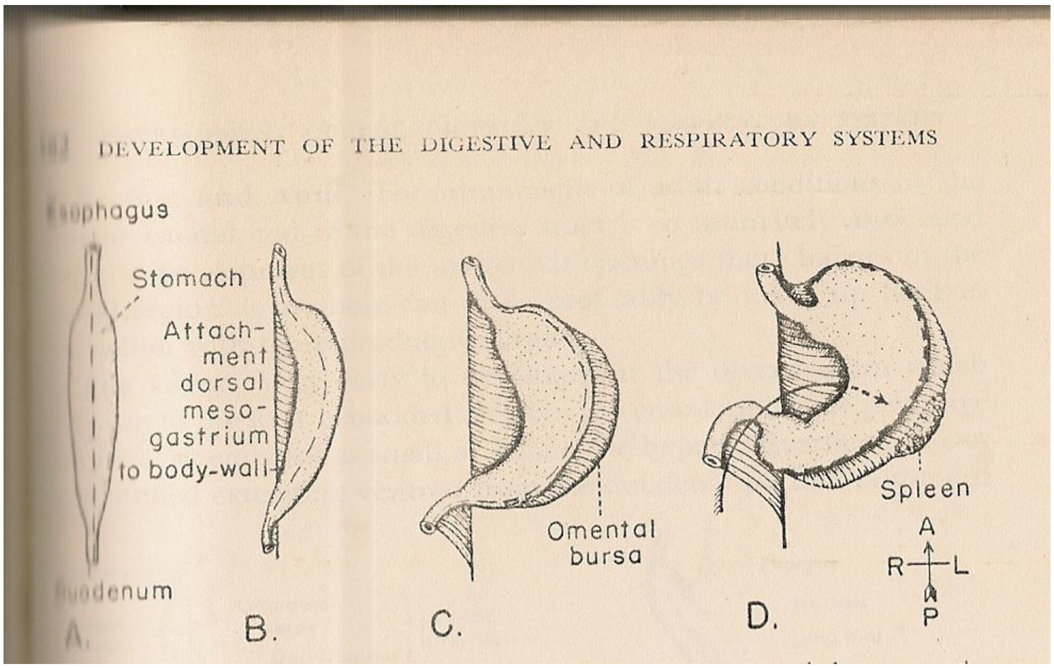
Caudal foregut

- The caudal foregut, sometimes simply called the foregut, begins after the respiratory diverticulum and extends to (and includes) the hepatocystic diverticulum.
- The derivatives of the caudal foregut include: esophagus, stomach, proximal duodenum, liver, gall bladder, hepatic and bile ducts, and pancreas.
- At first very short, the esophagus lengthens both prenatally and postnatally.
- At about 4 weeks, the stomach is a dilatation of the foregut.
- In the 5th week, the stomach rotates 90° around its longitudinal axis so that the original dorsal side becomes the left side.
- In the 6th week, the left side grows faster than the right, creating the greater and lesser curvatures.

- In the 7th week, further growth of the stomach gives the appearance of rotation around the dorsal-ventral axis, moving the distal foregut to the right.

- The liver bud (hepatocystic diverticulum) appears at the distal end of the foregut at 4 weeks; the hepatic and cystic diverticula, hepatic sinusoids and dorsal pancreas, at 4 ½ weeks; and the ventral pancreas, at 5 weeks. At 6 weeks, the pancreatic components meet and fuse.

Fig. 14



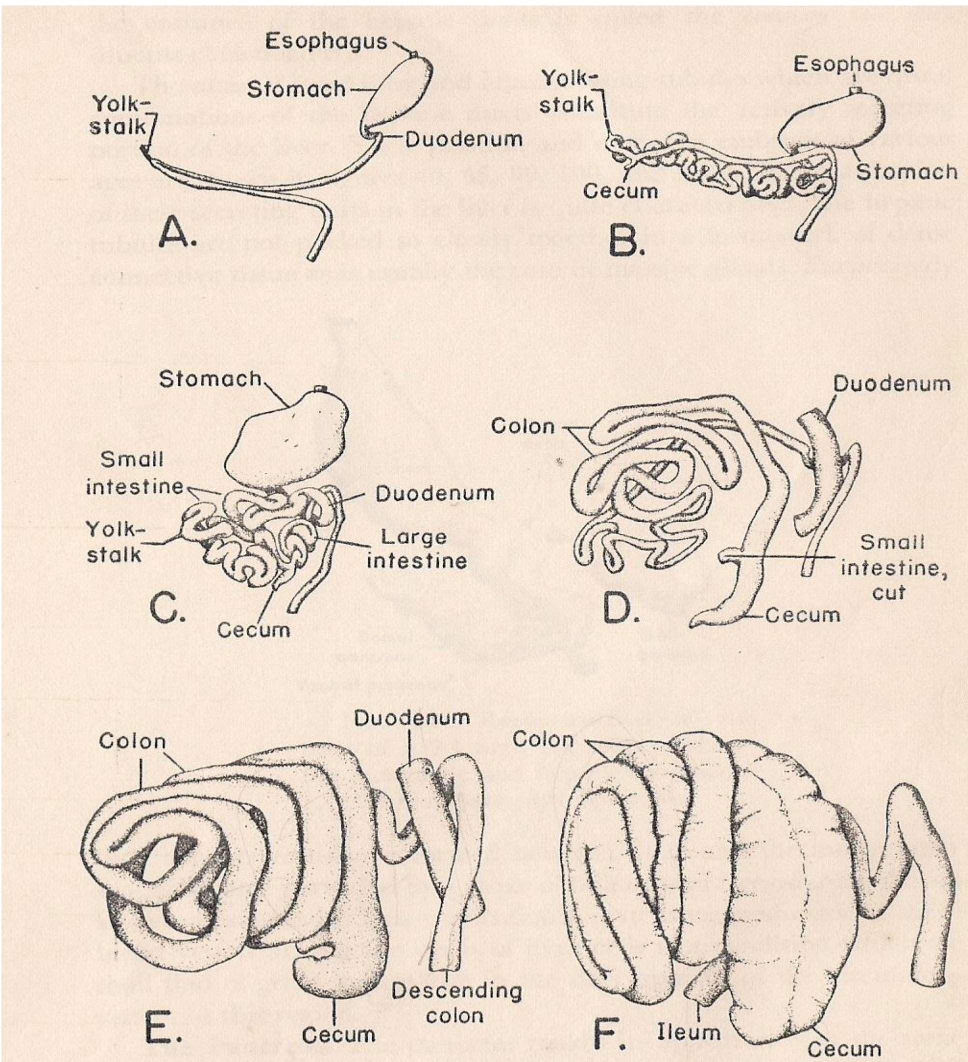
Development of the Midgut (weeks 5-10)

- The midgut is divided into two regions at the omphalo-enteric duct ("yolk stalk"): the cranial and caudal limbs.
- The derivatives of the cranial limb include the distal duodenum, jejunum, and proximal ileum.
- The derivatives of the caudal limb include the distal ileum, cecum, appendix, ascending colon, and proximal 2/3 of transverse colon.
- Proliferation of epithelium in many places in the developing GI tract may narrow the lumen during the 2nd month.
- The duodenum may be occluded during the 5th and 6th weeks. Recanalization of the duodenum in the 7th week is by coalescence of extracellular vacuoles. Failure of recanalization of the duodenum is believed to be a cause of duodenal atresia.
- The length of the midgut grows faster than that of the embryo, creating a gut "loop", with the omphalo-enteric (vitelline) duct at the apex. In the 6th week, the midgut enters the extraembryonic coelom of the developing umbilical cord (physiological umbilical herniation).

- At this time, the omphalo-enteric duct closes and the entire midgut loop rotates 90°. In the 10th week, the midgut returns to the abdomen, and rotates further (180° as measured at the apex of the loop).

After the midgut returns to the abdomen, there is shortening of some segments of the mesentery with fixation of three intestinal segments to the posterior abdominal wall. Fixation of the intestines results in most of the duodenum, pancreas, ascending colon and descending colon becoming secondarily retroperitoneal, and reduces the freedom of motion of the bowel, reducing the risk of volvulus and intestinal infarction.

Fig. 15



Development of Respiratory System:

- The respiratory system begins at the nasal cavity and consists of a conducting portion and a respiratory portion.
- The conducting portion includes nasal cavity, pharynx, larynx, trachea, bronchi, and bronchiole while the respiratory portion consists of the respiratory bronchioles, alveolar ducts, alveolar sacs and the alveoli.
- Gaseous exchange occurs in the alveoli.
- The development of the respiratory system involves the endoderm and the mesoderm that surrounds it.
- As discussed previously, the embryo undergoes lateral body folding and during this process the endoderm forms into a gut tube.
- This G.I. tube begins cephalically at the oral plate and continues until it reaches the cloacal or anal plate.
- If we look at the gut tube at the time the body folding is completed, it can be subdivided into three divisions: a foregut, a midgut and a hindgut.

Larynx

- The larynx is first seen as an outgrowth from the foregut.
- The outgrowth of tissue is called the **respiratory diverticulum** or the **lung bud**.
- The formation of the lung bud occurs when two lateral folds of splanchnic mesoderm and endoderm meet in the midline and separate the larynx and trachea from the esophagus.
- The lung bud is a ventral diverticulum of endoderm that arises from the floor of the foregut caudal to the pharynx.
- The diverticulum forms a groove in the floor of the pharynx called the **laryngotracheal groove**. Cephalic to the laryngotracheal groove is the **epiglottal swelling**.
- On either side of this groove are the developing **arytenoid swellings.**

Trachea

- **The trachea** develops caudal to the larynx.
- The epithelium develops from the endoderm and the tracheal cartilage and muscles develop from splanchnic mesoderm.
- Early in development the **trachea bifurcates** into the left and right bronchi.

Bronchi and Bronchioles

- As the bronchi develop they continue to branch.
- The right bronchus gives off three diverticula and the left bronchus gives off two diverticula.
- These diverticula become the **lobar bronchi** and indicate that the right lung will have three lobes and the left lung will have two lobes.

- Each of the bronchi at this stage will divide into smaller bronchi.
- The branching of the bronchi continues until the **bronchioles** begin to form. In all there are 17 divisions of the bronchi until the sixth fetal month is reached.
- However, by early childhood there will be a total of 24 generations of branching that occurs.
- As the lungs develop and divide into smaller divisions there are changes in the vascular supply of the lungs as well.

Fig. 16

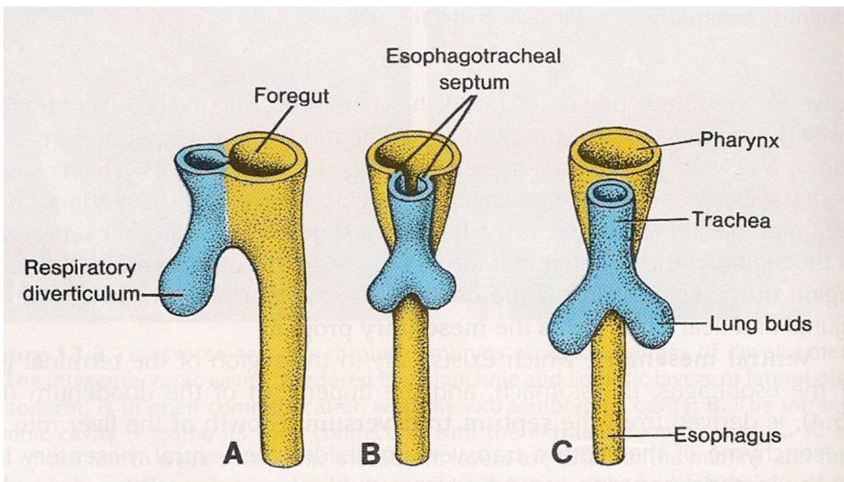
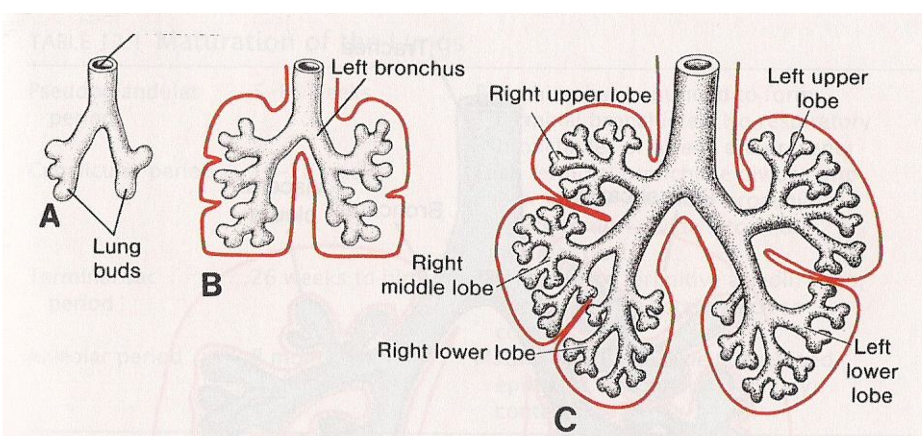


Fig. 17



Formation of Urinary System:

- Most of the urinary system develops from the intermediate mesoderm and the caudal hindgut during embryogenesis in the embryonic period.
- Derivatives of the intermediate mesoderm include the nephrogenic cord and the ureteric bud, which give rise to the kidneys and ureters, respectively.
- The urinary bladder and proximal urethra develop chiefly from the ventral portion of the cloaca.

Kidney and Ureter:
Development of the kidney and ureter can be divided into four stages:

Pre-nephric stage (weeks 1-5)

During the pre-nephric stage, the embryo produces no urine.

- At the end of the 4th week, the intermediate mesoderm is evident. It gives rise to the **nephrogenic cord** (urogenital ridge). This mesenchyme will give rise to the adrenal cortices, kidneys, ureters, gonads and reproductive tracts.
- In the 5th week, the nephrogenic cord undergoes segmentation, forming **nephrotomes**, which will become the **mesonephros**. (The caudal nephrogenic cord remains unsegmented.) Mesonephric ducts appear dorsolateral to the nephrotomes, which form mesonephric vesicles. The vesicles become glomerular capsules (invaded by capillaries) and tubules, and the ducts extend to reach the cloaca.
- Towards the end of the 5th week, a **ureteric bud** (metanephric diverticulum) appears on each mesonephric duct and grows to contact the caudal nephrogenic cord (**metanephric blastema**). Epithelio-mesenchymal interactions between the ureteric bud and nephrogenic cord are important in kidney development.

Mesonephric stage (weeks 6-10)

In the mesonephric stage, transient nephrons in the mesonephros produce urine.

- In the 6th week, the mesonephros begins to produce urine. In the metanephros, embryonic pelvis ("propelvis") and calices ("pseudocalices") appear.
- In the 7th week, the **metanephros**, which started in the sacral region, has ascended to the lumbar region (perhaps largely due to straightening of the body). The tips of the branches of the ureteric bud induce formation of vesicles in the metanephric blastema. The metanephric vesicles elongate and fuse with ampullae at the end of the ureteric bud. The vesicles develop into nephrons and the ampullae, into collecting tubules.
- By the end of the embryonic period, the mesonephros regresses but a few mesonephric tubules remain. (Some of these will become efferent ductules in males.)

Metanephric stage (fetal period)

By the end of the first trimester, the mesonephros ceases to be a functional kidney and the metanephros begins to produce urine. During the fetal period, the metanephros becomes the definitive kidney.

Bladder and Urethra

The urinary bladder and the proximal urethra are formed chiefly from the ventral portion of the cloaca. Thus the epithelium is endodermal in origin, and the muscle and connective tissue are from splanchnic (lateral plate) mesoderm. The trigone of the bladder may be formed by incorporation of the proximal segments of the mesonephric ducts into the wall of the bladder. The **distal urethra** is sexually dimorphic. The different fates in males and females of the definitive urogenital sinus, from which the distal urethra is derived, is described with the development of the external genitalia.

The cloaca and its division

- At the end of the 4th week, embryo folding produces the cloaca, the expanded end of the hindgut. A cloacal membrane marks the end of the hindgut. (In lower vertebrates, the cloaca is the common passage for fecal, urinary and reproductive excretions. In mammalian embryos, the cloaca is divided during development of the anorectum, bladder, urethra and genital systems.)
- In the 5th week, the allantoic diverticulum and mesonephric ducts join to the cloaca. The **urorectal septum** begins division of the cloaca.
- In the 6th week, the cloaca is divided into the **primary urogenital sinus** ventrally and the anorectum dorsally by the urorectal septum (which has not quite reached the cloacal membrane).

Development of the primary urogenital sinus

- The entry point of the mesonephric duct is used by anatomists to divide the primary urogenital sinus into the **vesico-urethral canal** cranially and the **definitive urogenital sinus** caudally.
- In the 7th week, the proximal mesonephric duct is incorporated into the dorsal wall of the vesico-urethral canal (the future trigone), so that the mesonephric and metanephric ducts enter separately. During further development, these duct openings appear to migrate so that the metanephric duct opening is cranial to the mesonephric duct opening. By the end of the 7th week, the cloacal membrane ruptures. The definitive urogenital sinus becomes continuous with the urethral groove.

Derivatives of the primary urogenital sinus

- The cranial portion of the vesico-urethral canal becomes the urinary bladder.
- The caudal portion of the vesico-urethral canal becomes most of the female urethra or the proximal half of the male's prostatic urethra.
- The fate of the definitive urogenital sinus is different in males and females.

Fig. 18

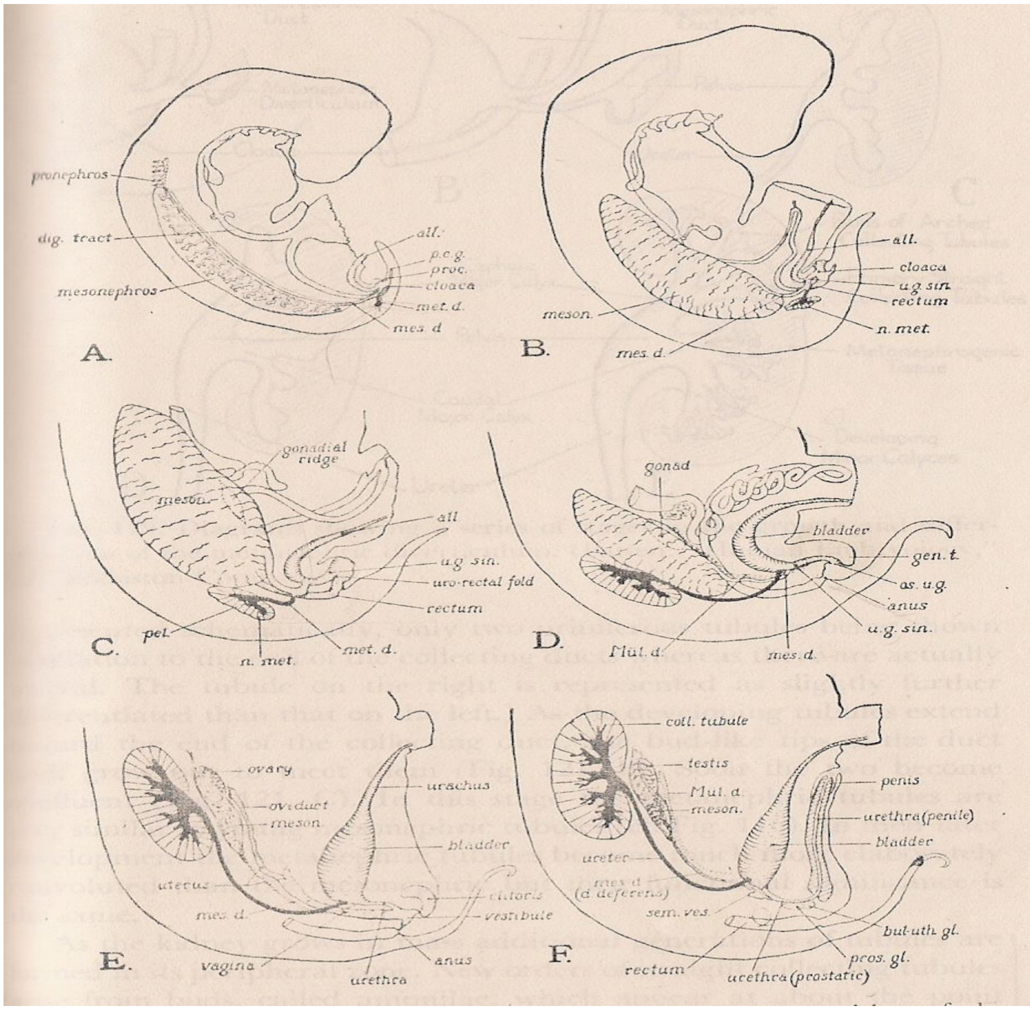
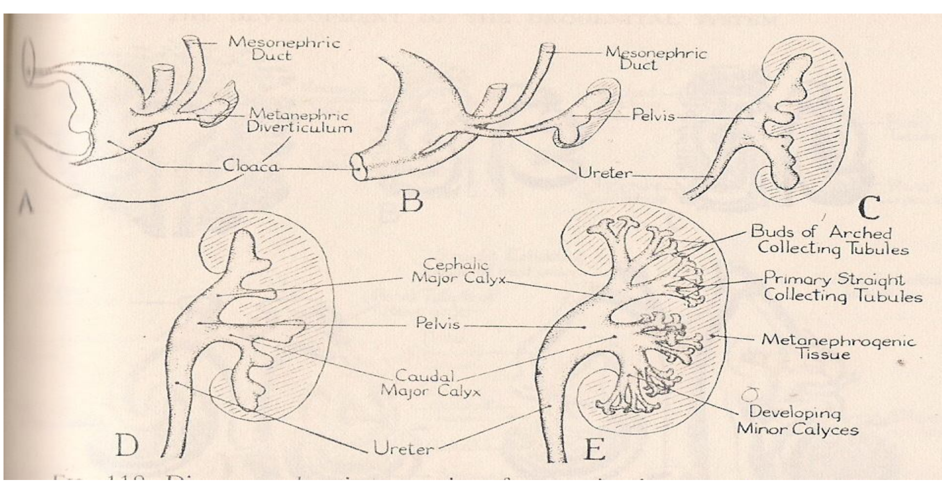

pronephros
dig. tract
mesonephros
all.
p.c.g.
proc.
cloaca
met. d.
mes. d
A.
all.
cloaca
u.g. sin.
rectum
n. met.
meson.
mes. d.
B.
gonadial ridge
meson.
all
u.g. sin.
uro-rectal fold
rectum
pel.
n. met.
met. d.
C.
gonad
bladder
gen. t.
os. u.g.
anus
u.g. sin.
Mul. d.
mes. d.
D.
ovary
oviduct
meson.
urachus
bladder
uterus
clitoris
vestibule
mes. d.
anus
vagina
urethra
E.
coll. tubule
testis
Mul. d
meson.
penis
urethra(penile)
bladder
ureter
mes. d (d. deferens)
sem. ves.
bulbuth. gl.
pros. gl.
rectum
urethra(prostatic)
F.

Fig.19

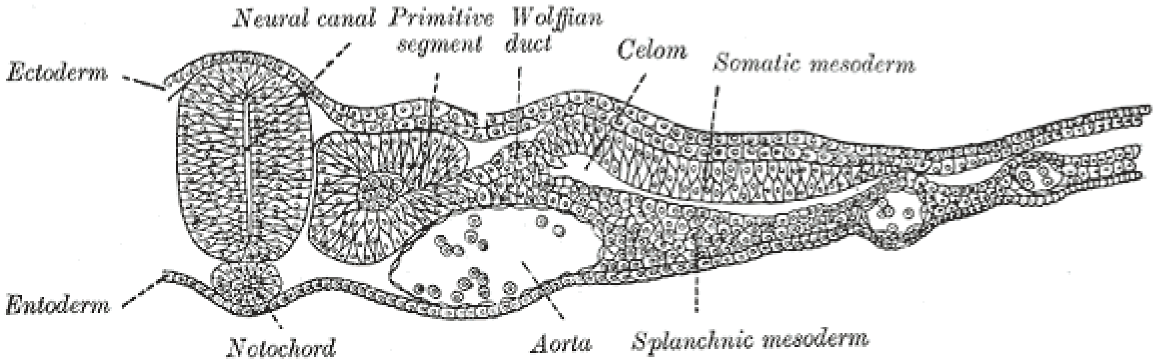

Mesonephric Duct
Metanephric Diverticulum
Cloaca
Mesonephric Duct
Pelvis
Ureter
B
C
Buds of Arched Collecting Tubules
Cephalic Major Calyx
Primary Straight Collecting Tubules
Pelvis
Metanephrogenic Tissue
Caudal Major Calyx
Developing Minor Calyces
D
Ureter
E

Development of Circulatory System:

Heart development

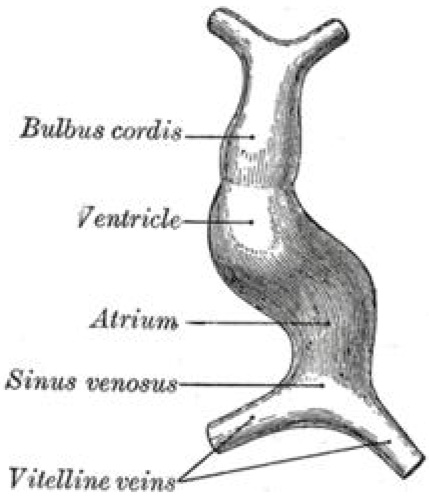
- The heart is the first functional organ in a vertebrate embryo. There are 5 stages to **heart development.**

Stage 1: Specification of cardiac precursor cells



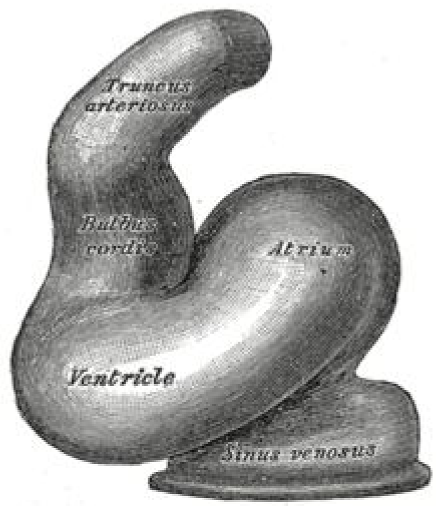
- The lateral plate mesoderm delaminates to form two layers:
  - The dorsal somatic (parietal) mesoderm and the ventral splanchnic (visceral) mesoderm.
- The heart precursor cells come from the two regions of the splanchnic mesoderm called the cardiogenic mesoderm.
- These cells can differentiate into endocardium which lines the heart chamber and valves and the myocardium which forms the musculature of the ventricles and the atria.
- The heart cells are specified in anterior mesoderm by proteins such as Dickkopf-1, Nodal, and Cerberus secreted by the anterior endoderm.

Stage 2: Migration of cardiac precursor cells and fusion of the primordia



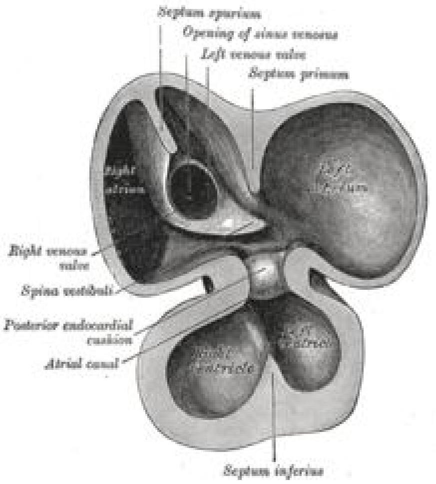
- The cardiac precursor cells migrate anteriorly towards the midline and fuse into a single heart tube.
- Fibronectin in the extracellular matrix directs this migration.
- If this migration event is blocked, cardia bifida results where the two heart primordia remain separated.
- During fusion, the heart tube is patterned along the anterior/posterior axis for the various regions and chambers of the heart.

Stage 3: Heart looping



- The heart tube undergoes right-ward looping to change from anterior/posterior polarity to left/right polarity.
- The detailed mechanism is unknown however the looping requires the asymmetrically localized transcription factor *Pitx2*.
- The direction of asymmetry is established much earlier during embryonic development, possibly by the clockwise rotation of cilia, and leads to sided expression of Pitx2. Looping also depends on heart specific proteins activated by Nkx2.5 such as *Hand1*, *Hand2*, and Xin.

Stage 4: Heart chamber formation



- The cell fates of the heart chambers are characterized before heart looping but cannot be distinguished until after looping.
- Hand1 is localized to the left ventricle while Hand2 is localized to the right ventricle.

Stage 5: Septation and valve formation

- Proper positioning and function of the valves is critical for chamber formation and proper blood flow. The endocardial cushion serves as a makeshift valve until then.