6.1. DEVELOPMENT OF MESODERMAL ORGANS

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In vertebrates, the mesoderm becomes partitioned at an early stage into four zones, from medial to lateral:
1. NOTOCHORD: occupies the midline.

2. PARAXIAL MESODERM: future somites.

3. INTERMEDIATE MESODERM: forms gonads, kidneys, and adrenals.

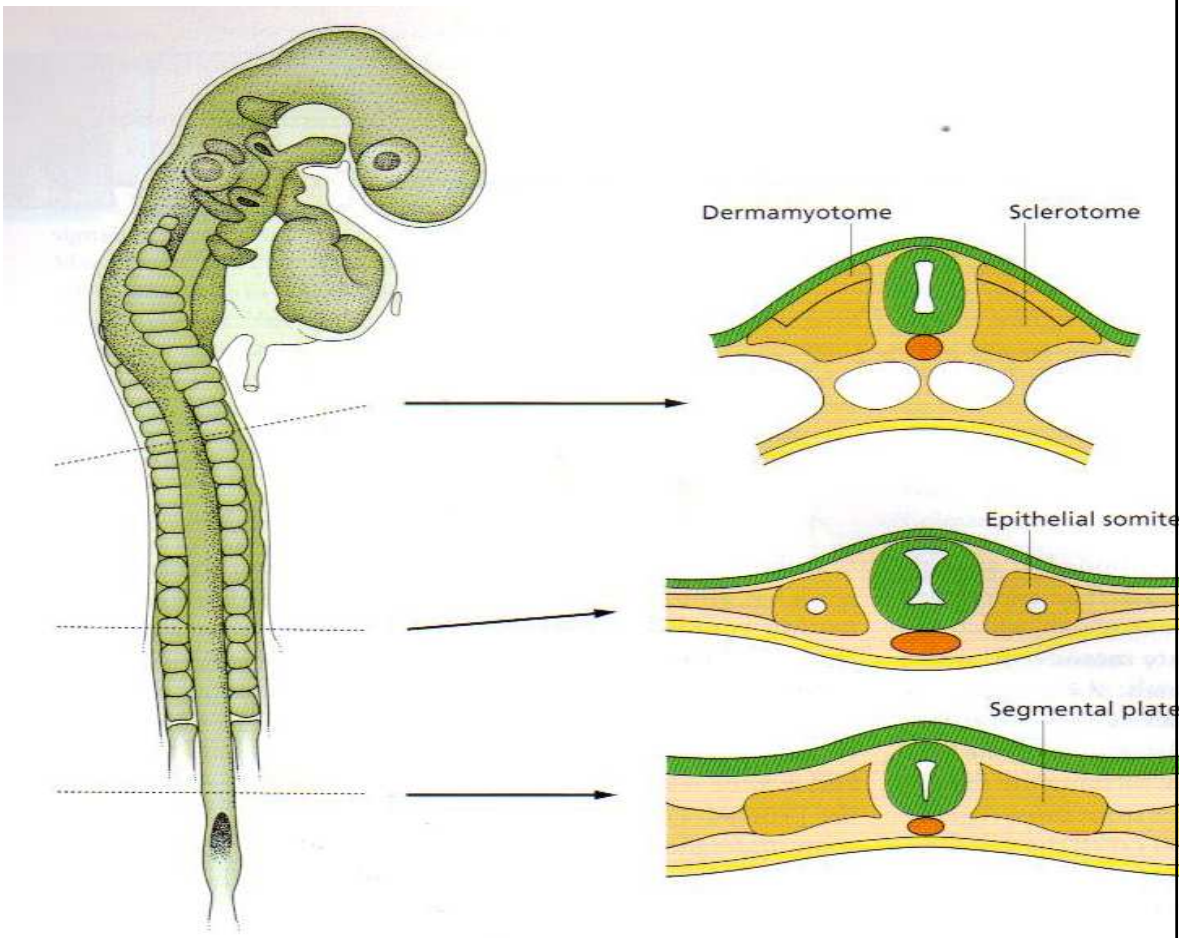
4. LATERAL PLATE MESODERM: the lateral plate is subdivided by the coelom into the outer SOMATIC MESODERM (future limb buds) and inner SPLANCHNIC MESODERM that forms mesenteries and heart.

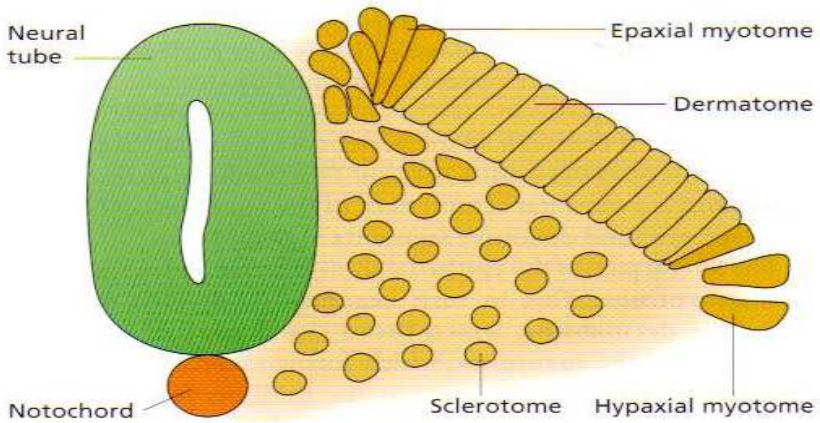
The skeleton originates from three regions: **Skull** is formed from **neural crest**; the **vertebrae** are formed from **somites**; and the **bones of the limbs** are formed from **limb buds** and associated **lateral plate**.

SOMITOGENESIS AND MYOGENESIS

Somite patterning is a clearest example of segmental arrangement of the vertebrate body. Somites arise in anteroposterior sequence from the **paraxial mesoderm** by the action of **forkhead** transcription factors **FoxC1** and **C2.**

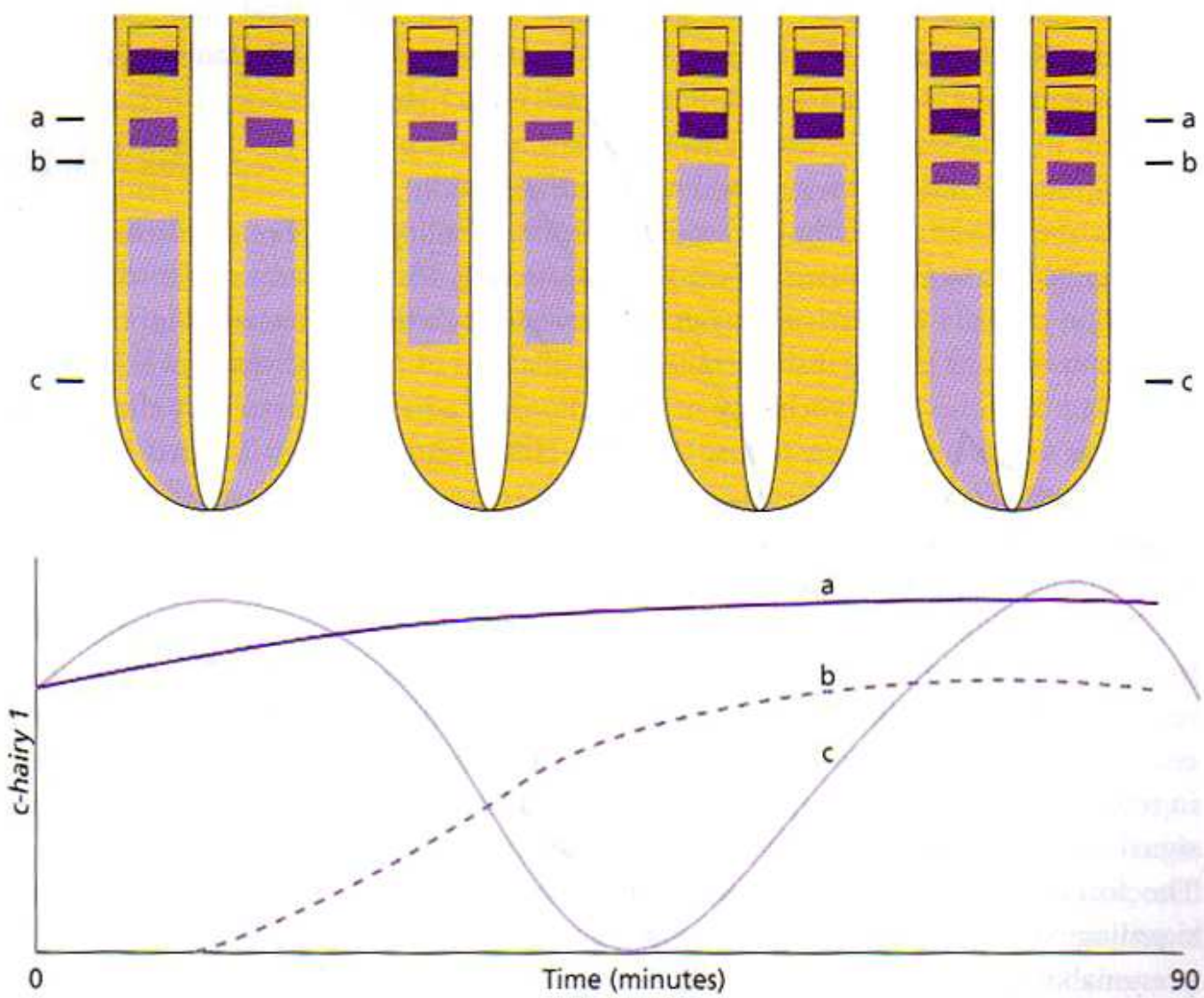
Somites start as loose cell associations called **somitomeres,** that later condense into the **epithelial somites**. This structure is transient as it undergoes epithelial-to-mesenchymal transformation to form the **sclerotome** (future vertebrae/ribs). Dorsal part of sclerotome forms **tendons** whereas the lateral part forms **dermamyotome** that later forms **skin** (dermatome) and **muscles** (myotome). Epaxial myotome forms segmental muscles of the body axis, hypaxial myotome forms muscles of the ventral body wall, limbs, and diaphragm.



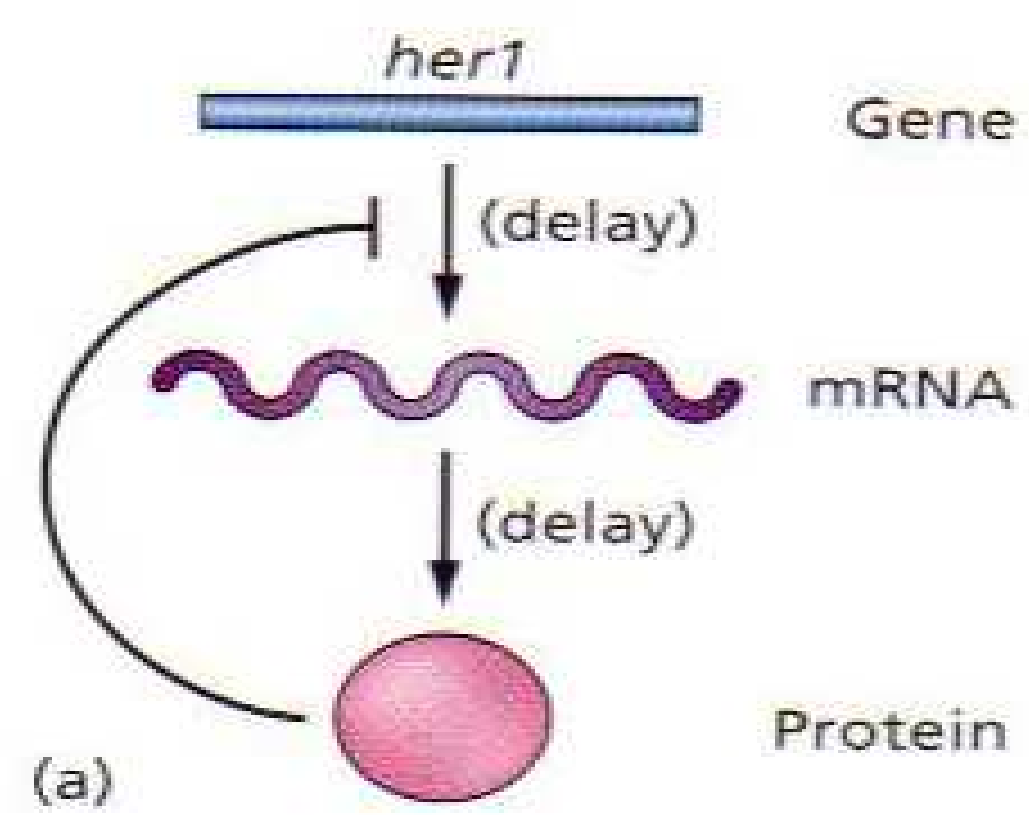


SEGMENTATION MECHANISM: Somites generated by molecular oscillator (a clock) operating in conjunction with a spatial gradient. One cycle of the clock forms one somite, the gradient determines that the somites are formed in anterior to posterior sequence. The clock represents a periodic expression of *c-hairy 1*, that encodes for transcription factor bHLH in chick.

Operation of somite oscillator over one cycle of somite formation. The diagrams show the expression pattern of c-hairy at four times of the cycle and the graphs show how the level of transcripts varies at the pointa a, b and c.



The actual oscillation is controlled by autoregulation of transcription of the hairy-type of transcription factors (her1 in mouse), through a negative-feedback mechanism.

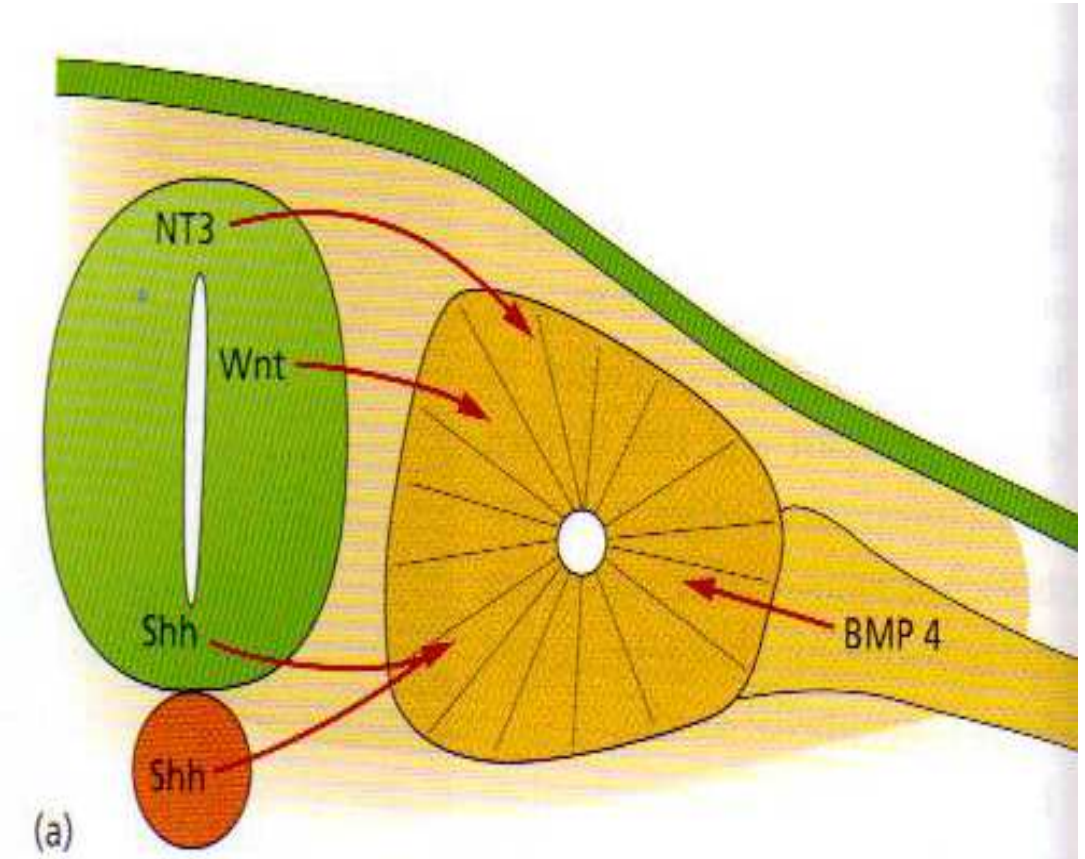


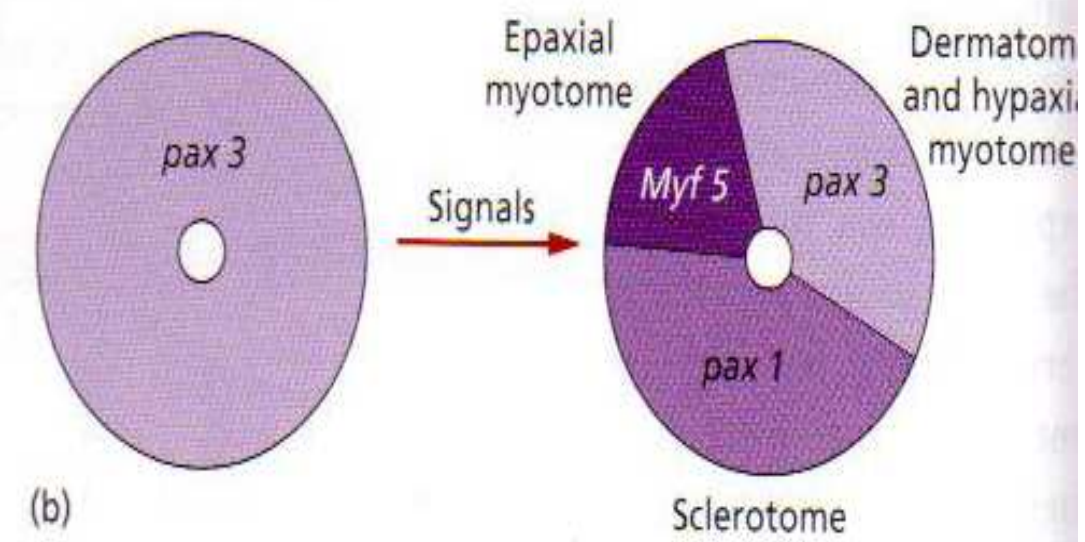
SUBDIVISION OF THE SOMITE: The subdivision of the somite depends on the interaction wiuth the surrounding tissues. The **sclerotome** is induced by notochord and ventral part of the neural tube. The **epaxial myotome** is induced by notochord and dorsal neural tube. The **dermatome** is induced by neural tube.

The signal for **sclerotome** induction is **Sonic hedgehog** (Shh).

The induction of **myotome** requires early exposure to **Shh** followed by **Wnt** signal from the dorsal neural tube. This results in the induction of the myogenic genes like *myf5* and regional repression of the myogenesis inhibitor *pax3*. The lateral plate-derived BMP4 represses myogenesis to balance the system.

The **dermatome** arises because of the **neurotropin 3** signal from the neural tube.





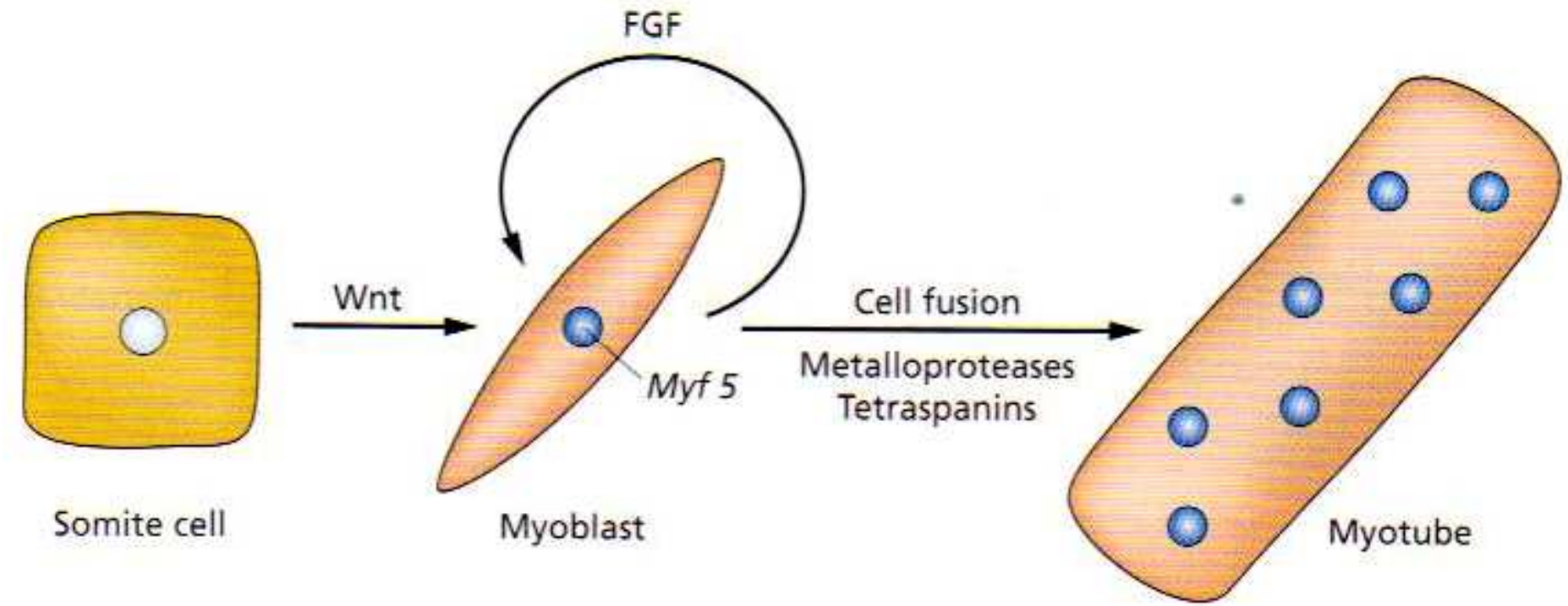
MYOGENESIS

SKELETAL MUSCLE: derived from myotome of the somites.

SMOOTH MUSCLE: formed in lateral plate mesoderm.

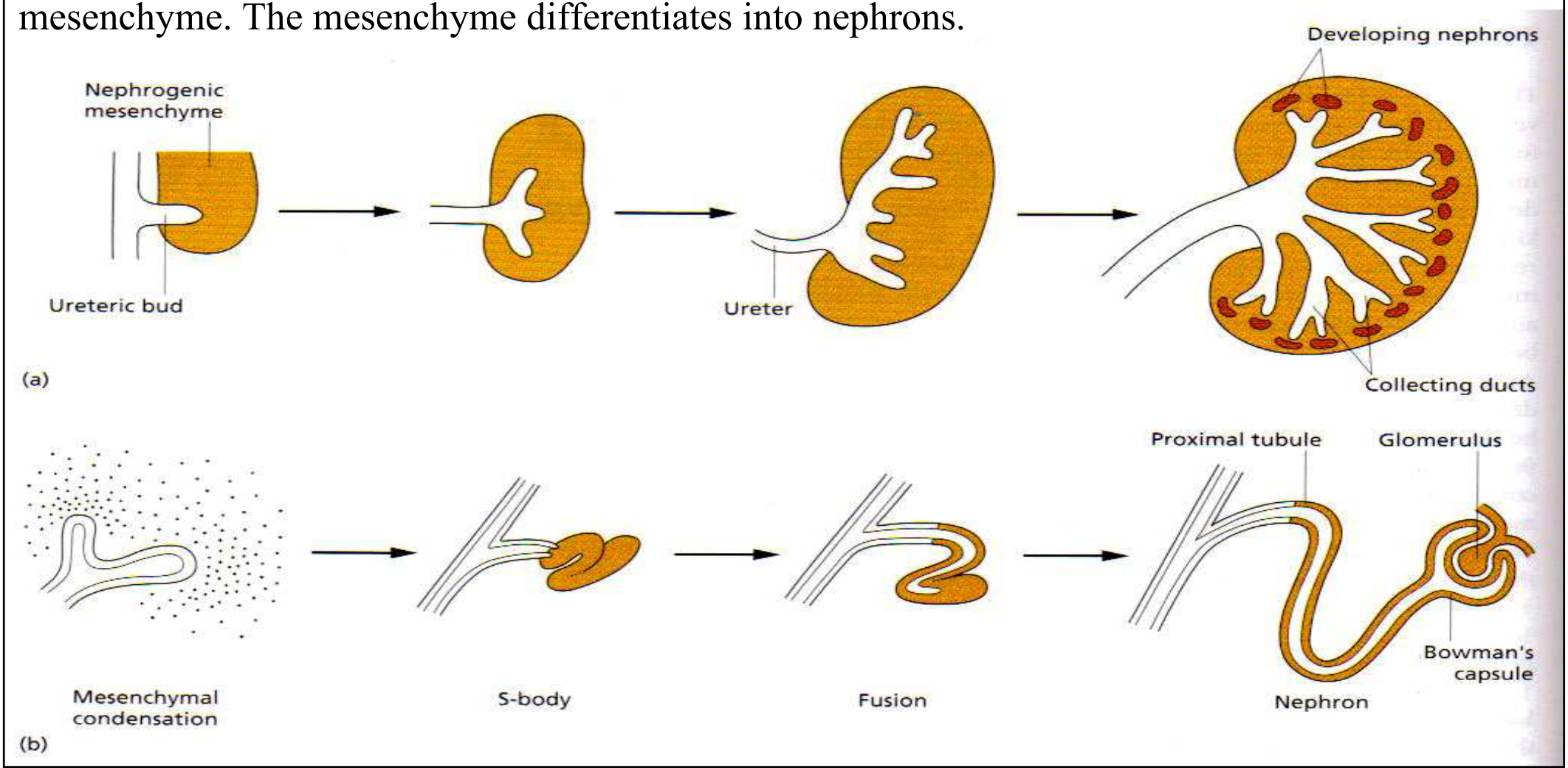
CARDIAC MUSCLE: formed in the myocardium of the early heart.

Skeletal muscle differentiation: cells commited to myoblasts diviode, migrate and fuse to form the multinucleate myofibers. **Myogenic proteins** – bHLH class of transcription factors (MyoD, Myf5, myogenin) capable of turning cells into the myogenic cells.



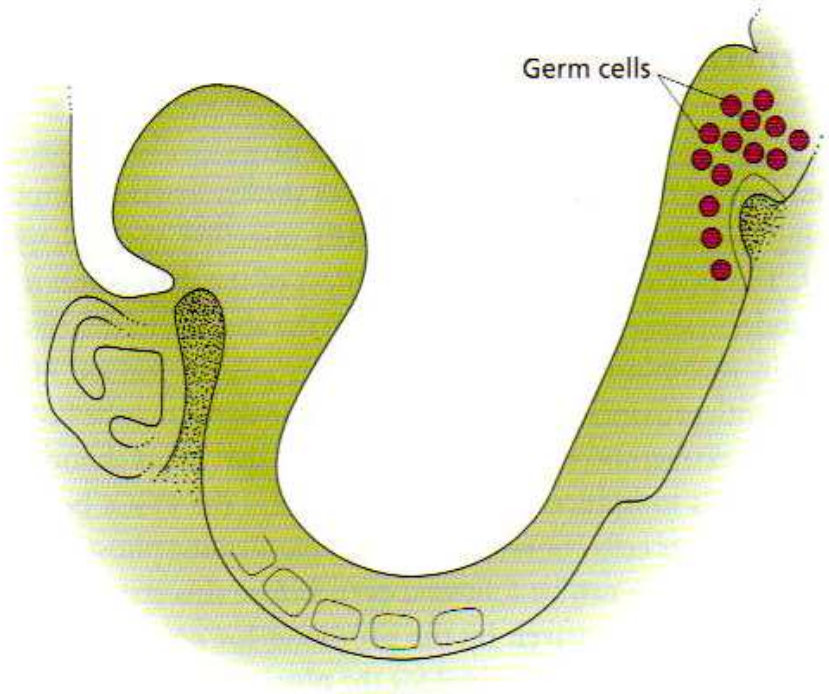
THE DEVELOPMENT OF THE KIDNEY

Kidneys originate from **intermediate mesoderm** (lateral to paraxial mesoderm). Amniotes has three kidneys – **pronephros** (non functional), **mesonephros** (functions transiently in embryonal life) and **metanephros** (definitive kidney). The **nephric duct** (Wollfian duct) originates in the anterior of intermediate mesoderm surrounded by the tissue committed to be kidney – **nephrogenic** mesoderm. The collecting system of metanephros is hovewer not formed from the main nephric duct, but from an outgrowth called **ureteric bud**, that growths into the nephrogenic mesenchyme. The mesenchyme differentiates into nephrons.

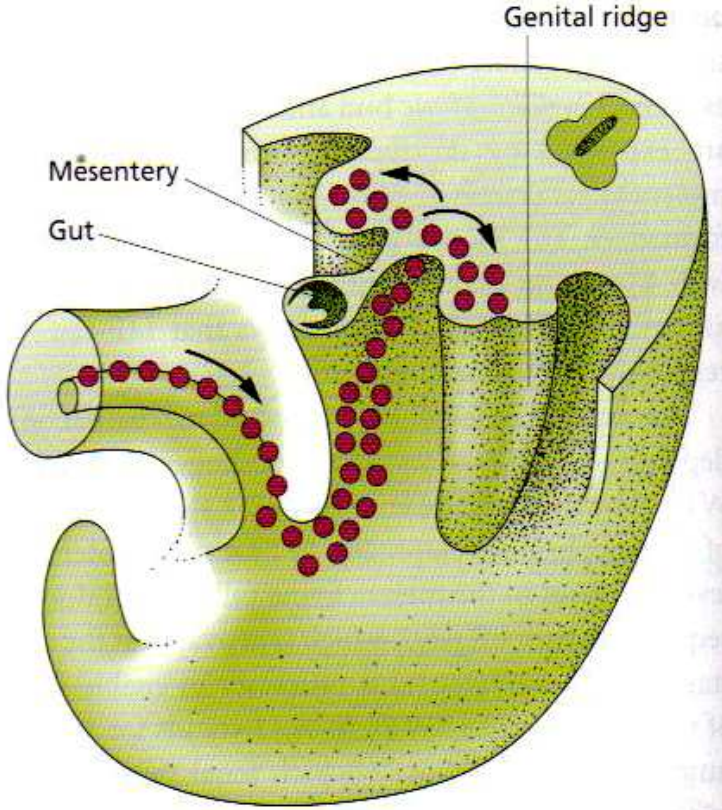


GERM CELL AND GONADAL DEVELOPMENT

Gonads have dual origin. The somatic tissue originates from **genital ridges** of the **intermediate mesoderm.** The germ cells are derived from **primordial germ cells (PGCs)** that migrate to the genital ridges from the **proximal part** of the **egg cylinder**. PGCs are induced by the **extraembryonic ectoderm** via **BMP4** signal. The migration of PGCs is driven by chemokine **SDF1** (stromal cell derived factor 1) produced by lateral plate mesoderm.

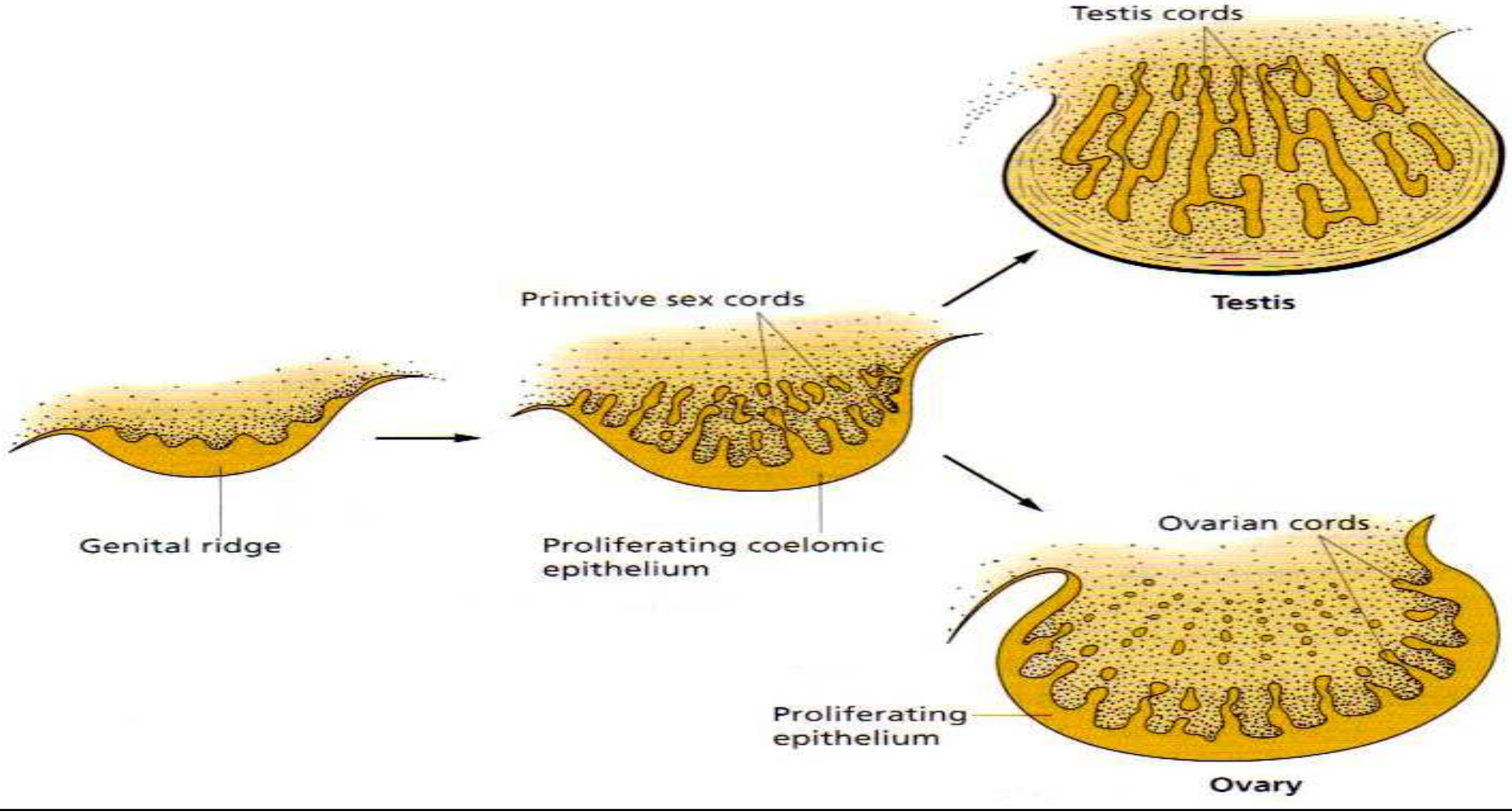


8.5 day

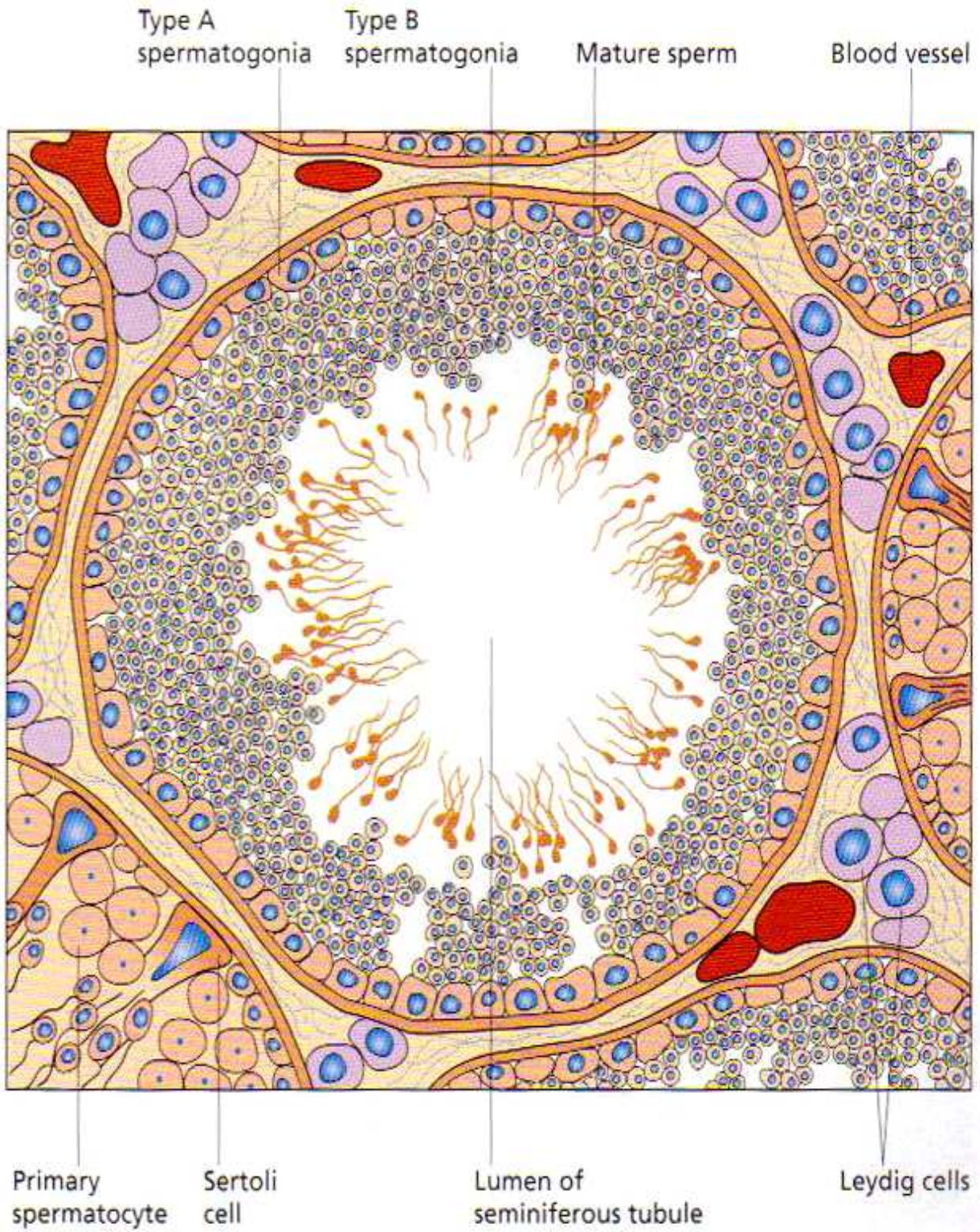


10.5 day

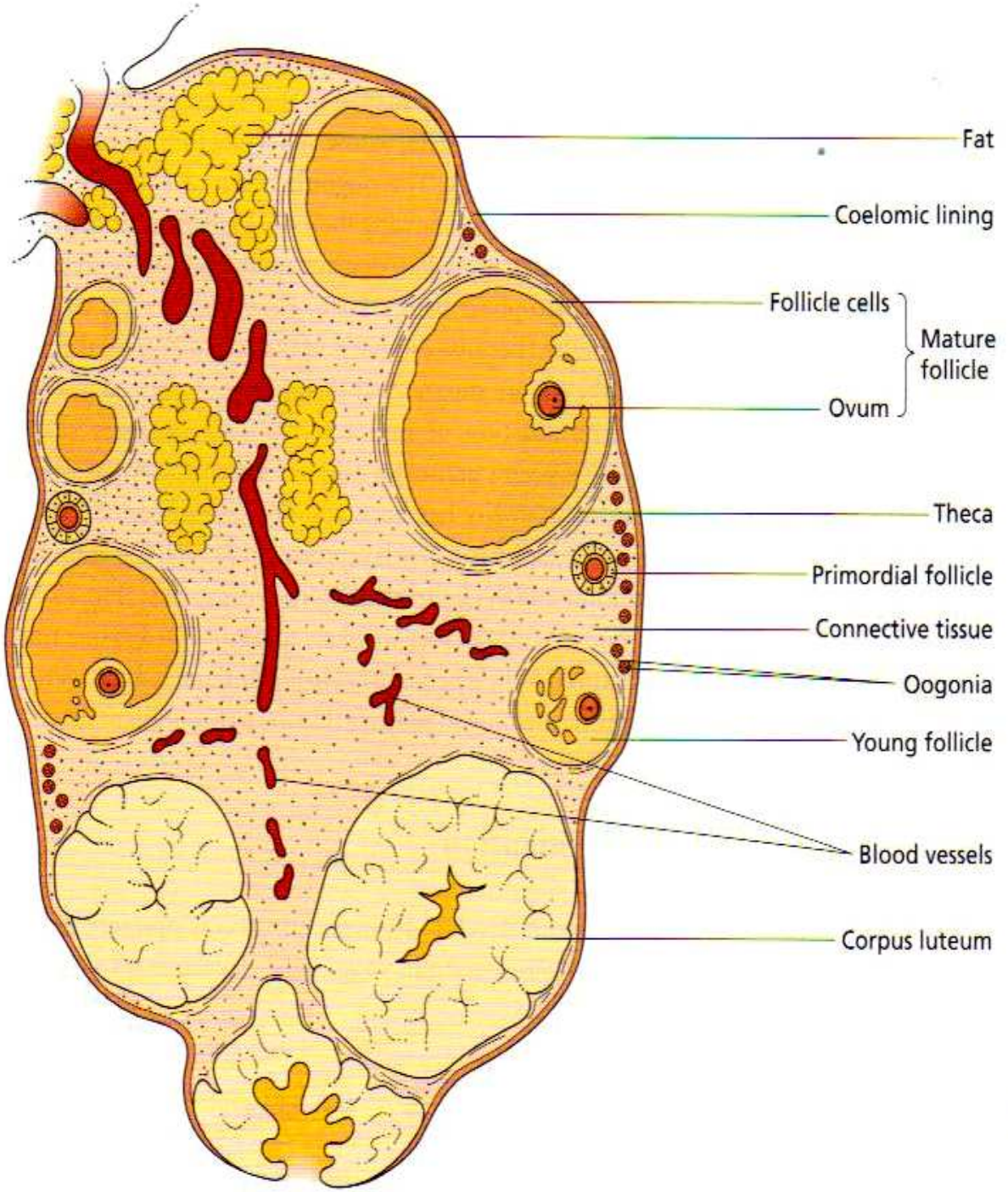
Gonad development depends on sex determination. Gonads develop from the **medial part** of **intermediate mesoderm** that forms genital ridge at 9.5E in mouse. Shortly after the appearance of the genital ridge, cord of cells begin to form from the coelomic lining epithelium and grow into the underlying mesenchyme. Two ducts arise in this period, the **nephric duct** and the **Mullerian duct** that will eventually became the oviduct, uterus and proximal vagina of a female. There is no difference between two sexes by this stage (E12.5 in mouse).



In male, the cords of coelomic lining cells form a complex systém of **seminiferous tubules** composed of **Sertoli cells** into which the germ cells are integrated. Cells responsible for testosteron production (**Leydig cells**) differentiate from mesenchyme between the tubules. The tubules became connected to **nephric duct** and the **Mullerian duct** regresses. The tubules remain solid until after birth, when they start to hollow out and germ cell-derived **spermatogonia** appear.



In the female, the cords of cells derived from coelomic lining stay near the surface as **granulosa** (follicle) cells, in proximity to the to the **oogonia**. **Thecal cells** that produce the **estrogen** differentiate from the mesenchyme. The gonad becames encapsulated as ovary and the **nephric duct** degenerates. The two Mullerian ducts fuse at their posterior ends to forma proximal part of vagina and uterus.

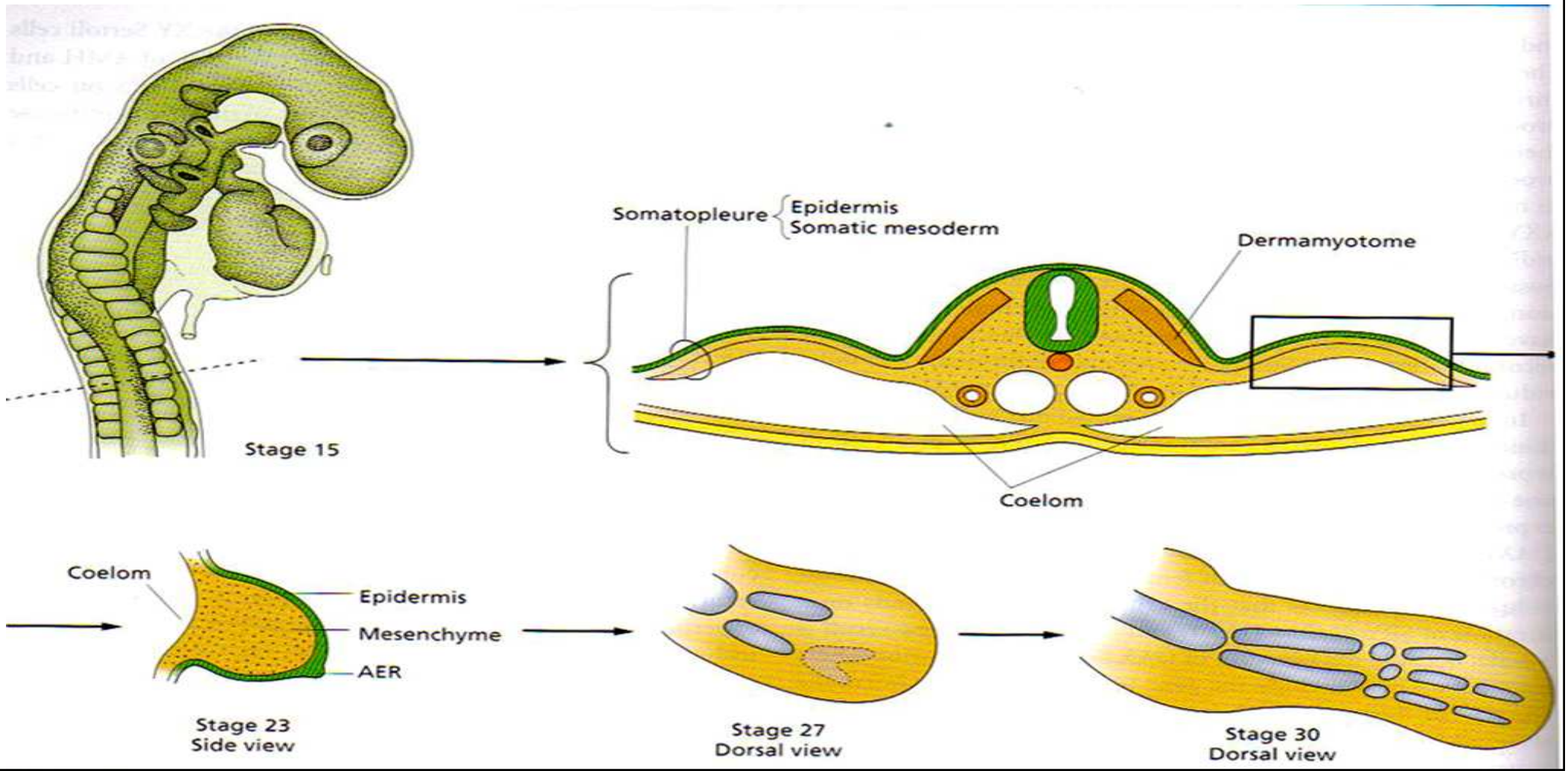


SEX DETERMINATION: Gonadal develoment does not require the presence of germ cells. **WT1** (zinc-finger transcription factor) and **SF1** (member of nuclear hormone receptor family) are essential to early, uniform development of gonad. The sex is determined by chomosomal constitution, with Y-linked **SRY** (sex determining region of Y, encodes for transcription factor of HMG group) being a critical switch controlling the process. If SRY is deleted from Y chromosome, the XY mouse develops as a female and *vice versa*.

SRY regulates sex development through repressing **DAX1**, a nuclear hormone receptor that inhibits various male functions like **AMH** (anti-Mullerian hormone) made by Sertoli cells.

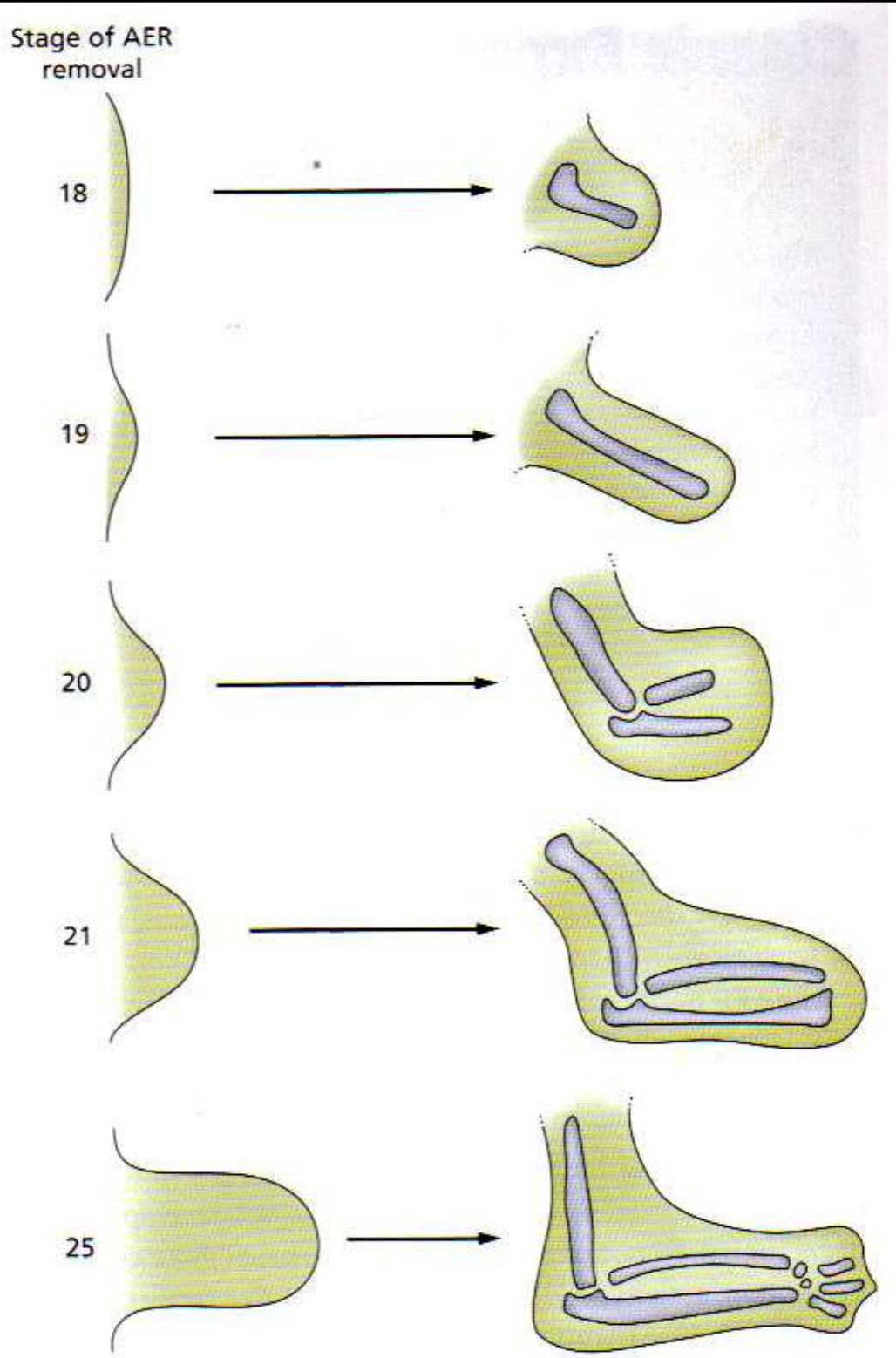
LIMB DEVELOPMENT

The limbs arise from **somatic mesoderm** (**somatopleure** = somatic mesoderm + overlying epidermis) that lies lateral to the intermediate mesoderm. The early limb bud is consisted of undifferentiated mesenchyme covered by epidermis, that is thickened to form the **apical ectodermal ridge** (**AER**) at the distal edge. The mesenchyme forms cartilage, tendons, ligaments, dermis and the sheats surrounding the muscles, the somites form the muscles themselves.

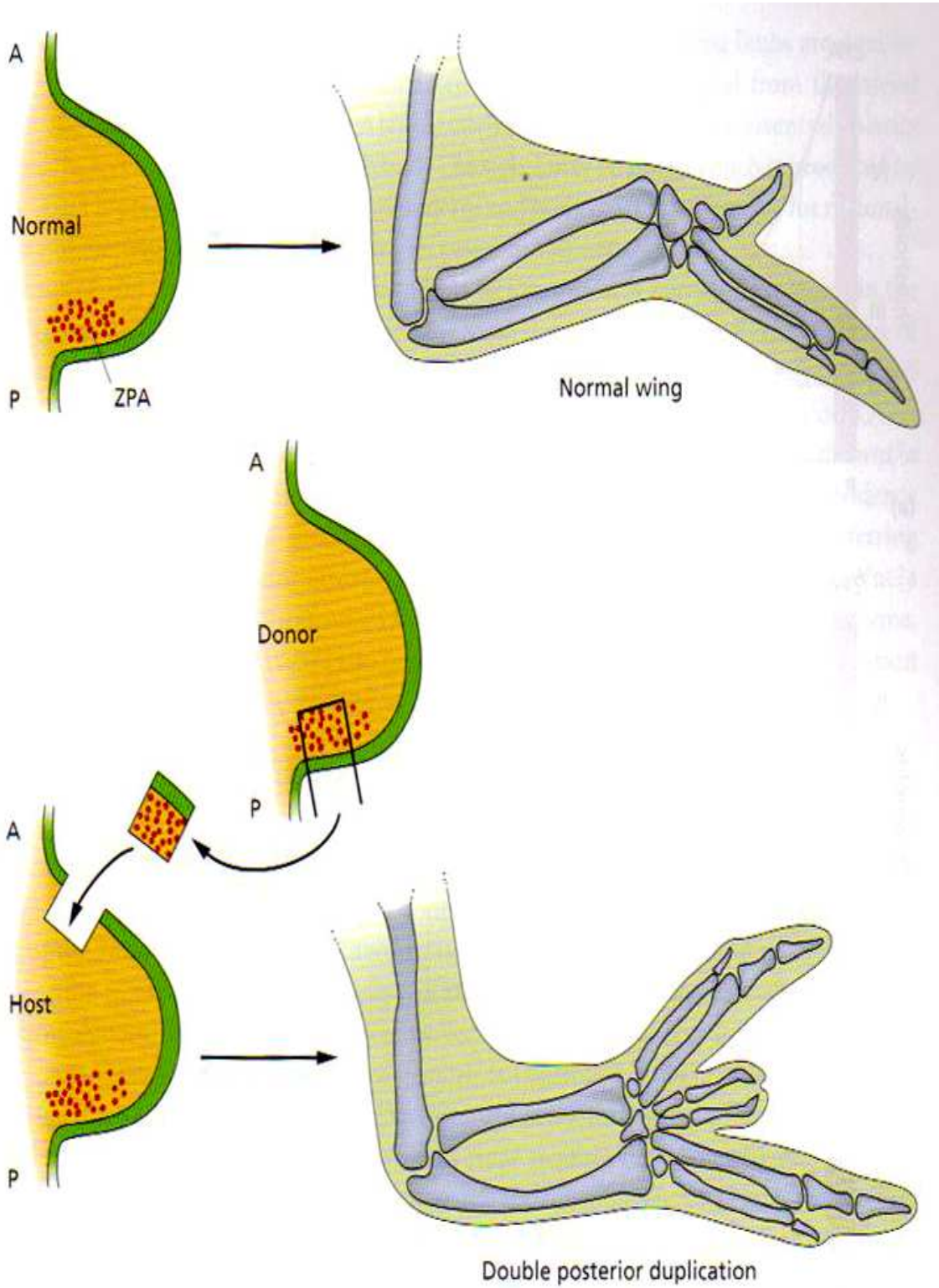


PROXIMAL-DISTAL OUTGROWTH AND PATTERNING: The earliest visible event is in limb-bud development is AER formation in the epidermis. The AER expresses transcription factors **Msx1** and **Msx2,** which is activated by **BMP** (Bone Morphogenetic Protein) signaling. BMP signaling is critical for AER formation.

The AER releases various factors to regulate the limb bud growth. These include **fgfs** (fibroblast growth factors), **Shh,** and **BMP**.

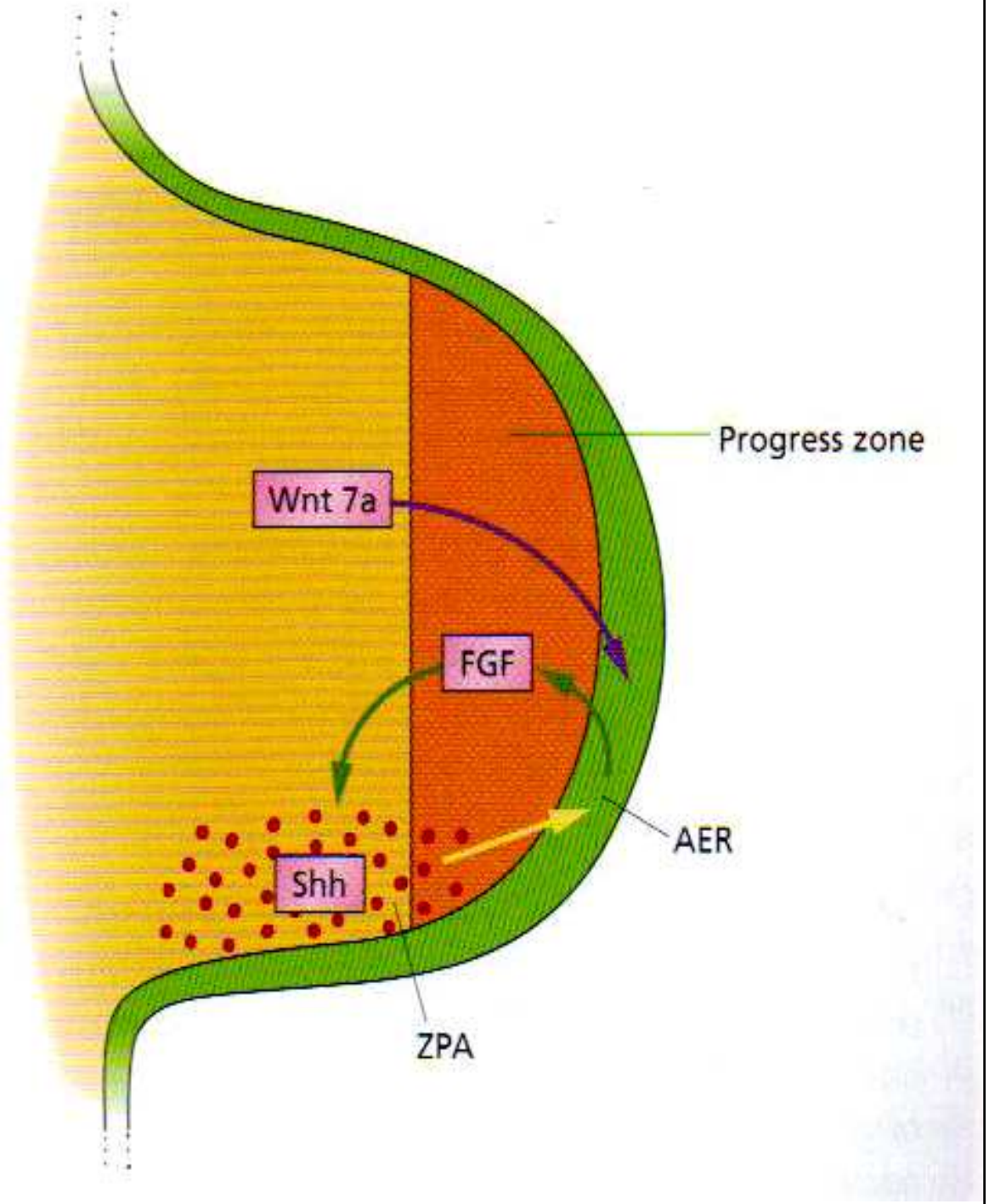


ANTEROPOSTERIOR PATTERNING: This pattern is controlled by **zone of polarizing activity** (ZPA) located at the posterior margin of the bud. ZPA is a source of the diffusible morphogen (**Shh**), that forms a gradient over the surrounding tissues. Shh regulates this stage of limb development through stabilizing **Gli transcription factor**.



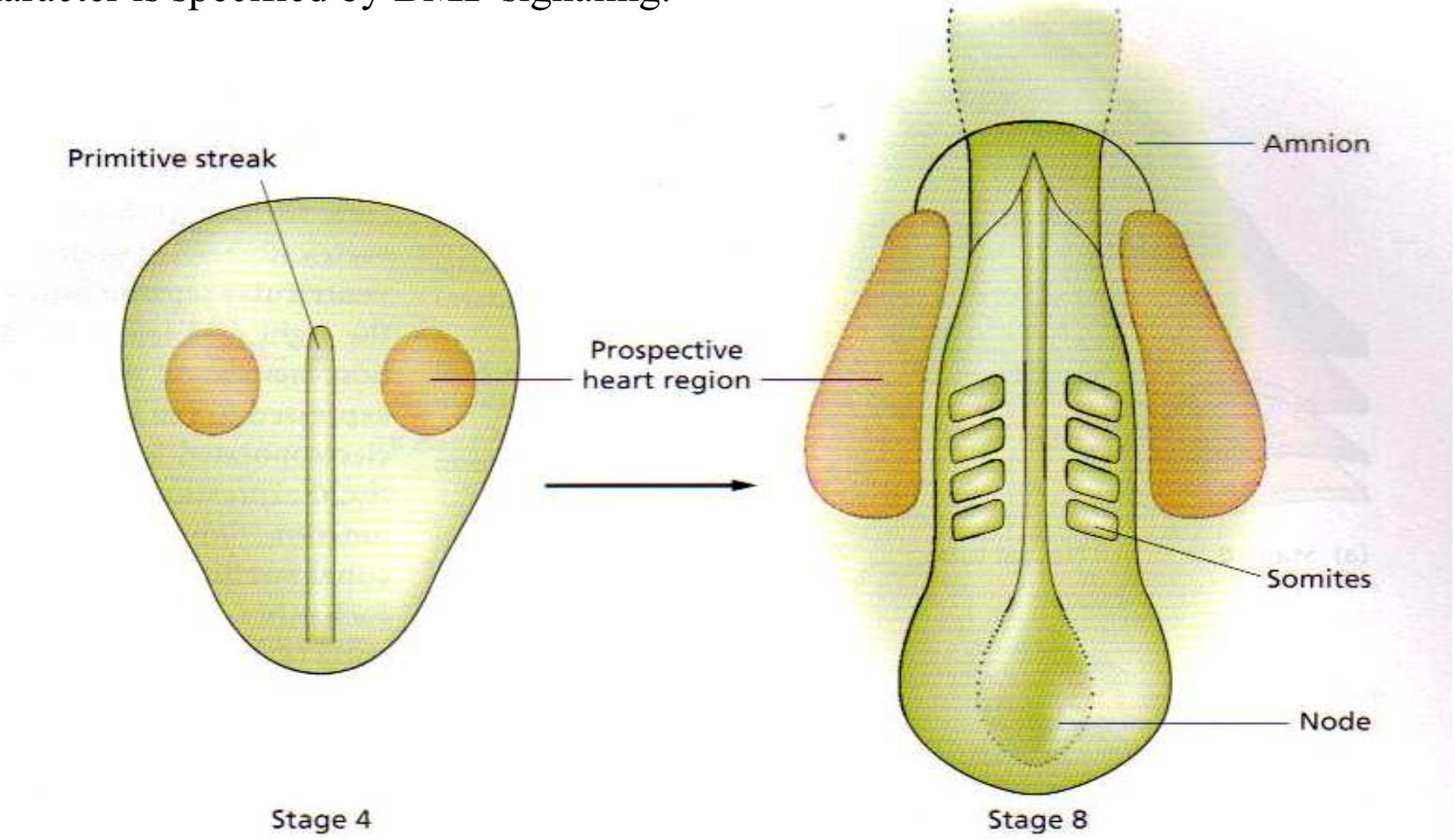
LIMB PATTERNING OVERALL:

Pattern in all three axes is controled by a separate process. **Proximal-distal** patterning relies on **FGF** supply from **AE**R. The **anteroposterior pattern** is controled by **Shh gradient** from **ZPA**. The **dorsoventral** pattern is controlled by **Wnt7** from the **dorsal epidermis**. The three processes work together to shape the organ, as evidenced by the fact that AER needs both Shh and Wnt7 for its survival and function.

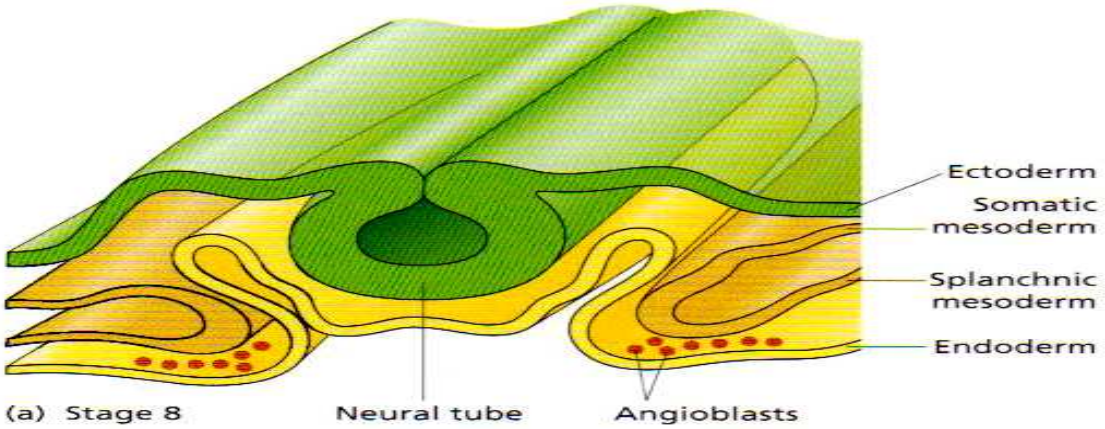


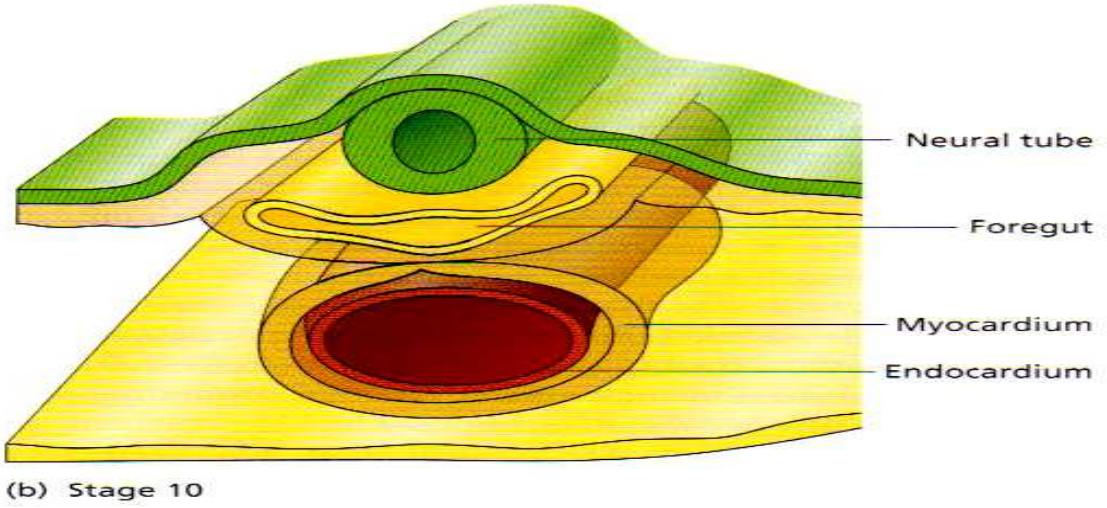
HEART DEVELOPMENT

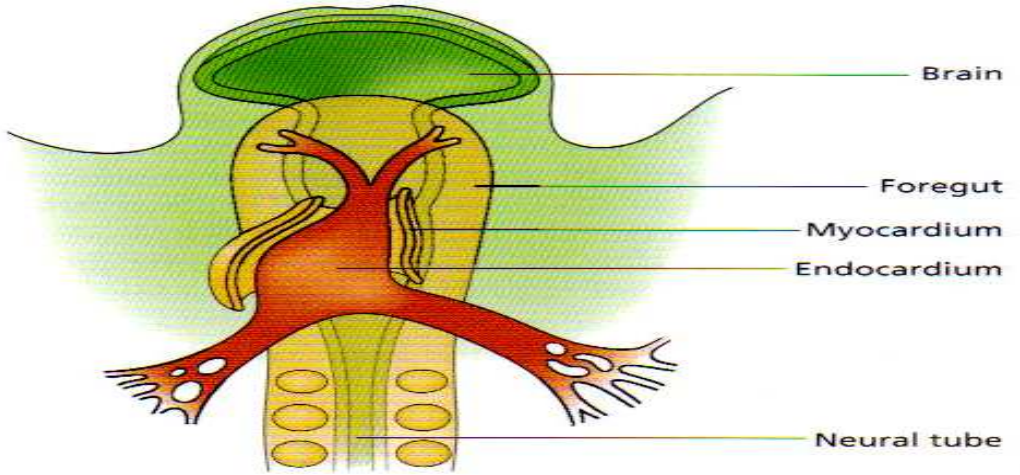
Cardiogenic mesoderm originates from **epiblast** lateral to the node in chick, later forming two elongated strips on either side of the embryonic axis. The early heart commitment of this region requires transcription factors **Nkx2.5**, **GATA4-6**, **MEF2** and **Tbx5**. Heart represents the **anteroventral sector** of the embryo body that is further specified by inhibition of **Wnt** and **Nodal** signaling by the action of factors as **Cerberus** and **Dickkopf**, while ventral character is specified by **BMP** signaling.



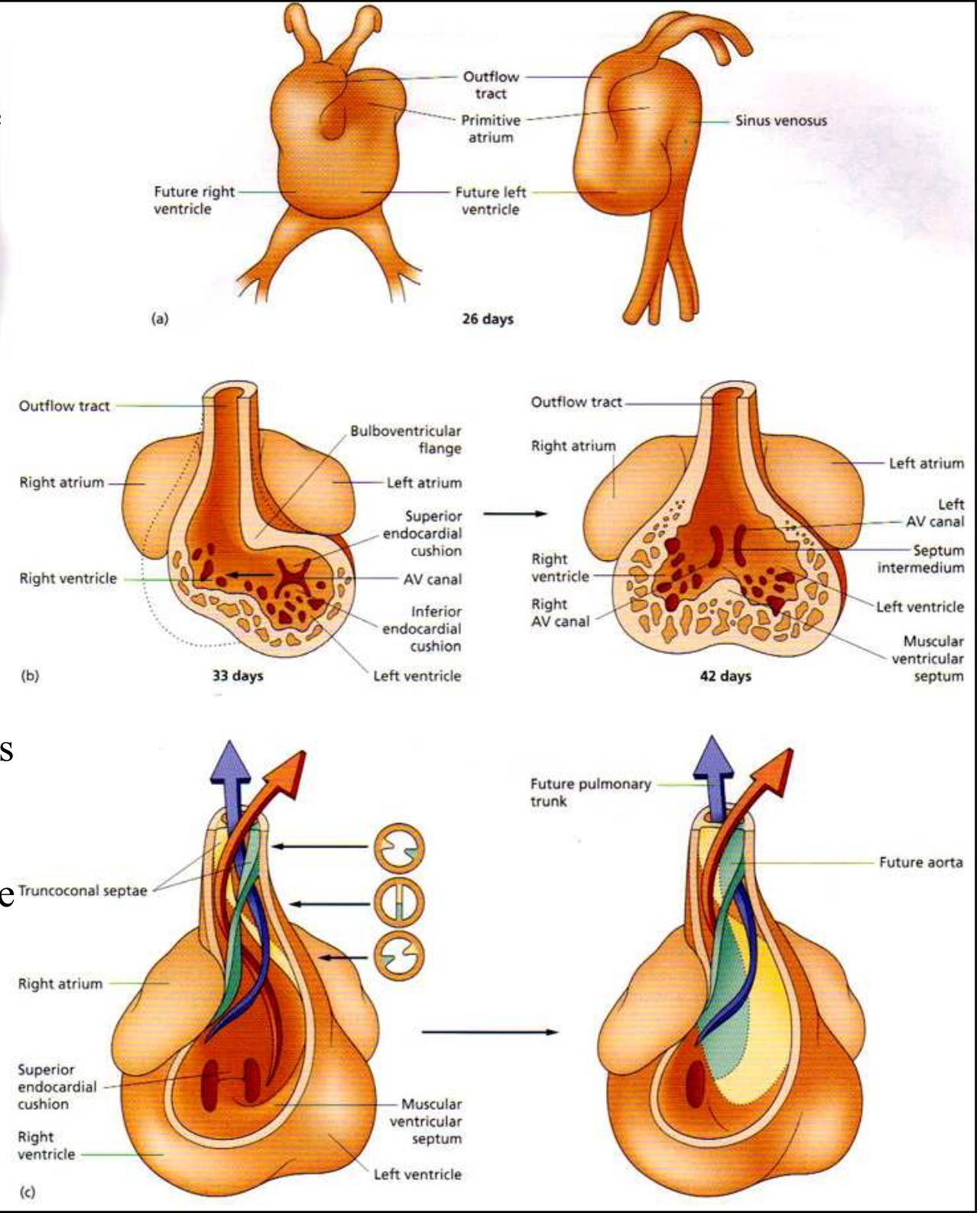
As the heads lifts off the blastoderm surface, the heart rudiments move underneath it towards the midline. This migration depends on **FGF8** and **fibronectin**. A failure of this migration leads to **cardia bifida**, where two separate hearts form side by side. Normally, both rudiments fuse to form a tube that has 4 layers: **endocardium**, **cardiac jelly**, **myocardium** and **pericardium**. Shortly after the fusion of two rudiments the heart tube starts pulsation.







The forming of heart in mammals involves conversion of a simple tube into the four-chambered heart with separate righ- and left-sided circulation. This involves looping, asymmetrical growth, movement of blood vessel insertion sites and formation of internal septa to divide the lumen. 1. Looping brings the atria to the anterior and ventricles to the posterior. 2. The venous returns run into the right side of the atrium and new pulmonary vein sprouts from the left side of the atrium. 3. Atrial septa and endocardial cushions form. 4. The cushions meet to form the septum intermedium dividing ventricle into left and right sides. The other cushions later contribute to the atrioventricular valves. 5. Muscular ventricular septum growths from ventricular wall to separate the right and left sides.



BLOOD VESSEL DEVELOPMENT

Vasculogenesis = *de novo* formation of blood vessels in embryo. **Angiogenesis** = formation of new capilaries from the existing ones by cell division and migration. Vasculogenesis is associated with the formation of blood since both vein endothelium and blood have the same precursor – **hemangioblast** – located in the **lateral plate mesoderm**. The hemangioblast population is characterized by expression of **SC**L (stem cell leukemia transcription factor), **flk1** (receptor for **VEGF**, vascular endothelial growth factor), **FGFR1** (receptor for FGF) and **GATA-1** and **-2** transcription factors. VEGF represents the most potent angiogenic factor, *vegf*$^{-/-}$ mice have no blood vessels.