Section 1: Embryological Development of the Lower Limb.

Learning Objectives.

At the end of this section, you should be able to:

1. discuss the major stages of embryological development of the lower limb
2. discuss the development of the neurological supply to the lower limb
3. discuss the development of the vascular supply to the lower limb.

Human development is continuous, beginning with fertilisation. Cell division, migration, apoptosis, differentiation and growth all contribute to the development of the zygote into a multi-cellular human being. Most changes occur during the embryonic and foetal periods, although further important changes take place at birth, childhood, adolescence, and early adulthood. Embryology refers to the study of the embryo, although generally it is used to refer to the study of prenatal developments.

This course aims to focus specifically on the development of the lower limb during prenatal development. Before any limb development, the embryo has undergone a series of changes and developments, and for the purposes of limb development, the formation of the three germ layers (arising from the embryonic disc) is essential. This noted by the third week, characterised by the appearance of the primitive streak, the development of the notochord, and differentiation of the three germ layers.
Gastrulation : Formation Of Germ Layers

Gastrulation is the process by which the three germ layers, which are precursors of all embryonic tissues, are established in embryos. During gastrulation, the bilaminar embryonic disc is converted into a trilaminar embryonic disc. Extensive cell shape changes, rearrangement, movement, and changes in adhesive properties contribute to the process of gastrulation. Gastrulation is the beginning of morphogenesis (development of body form) and is the significant event occurring during the third week. The first morphologic sign of gastrulation begins with formation of the primitive streak on the surface of the embryonic disc. During this period, the embryo may be referred to as a gastrula. Each of the three germ layers (ectoderm, mesoderm, and endoderm) gives rise to specific tissues and organs:

- Embryonic ectoderm gives rise to the epidermis, central and peripheral nervous systems, the eye, and inner ear, and, as neural crest cells, and to many connective tissues of the head.
- Embryonic endoderm is the source of the epithelial linings of the respiratory and alimentary (digestive) tracts, including the glands opening into the gastrointestinal tract and the glandular cells of associated organs such as the liver and pancreas.
- Embryonic mesoderm gives rise to all skeletal muscles, blood cells and the lining of blood vessels, all visceral smooth muscular coats, the serosal linings of all body cavities, the ducts and organs of the reproductive and excretory systems, and most of the cardiovascular system. In the trunk, it is the source of all connective tissues, including cartilage, bones, tendons, ligaments, dermis, and stroma of internal organs.
KEY LEARNING POINT.

All connective tissues, including cartilage, bones, tendons, ligaments, and skeletal muscle arise from the embryonic mesoderm.
**Primitive Streak**

The first sign of gastrulation is the appearance of the primitive streak. At the beginning of the third week, the primitive streak appears on the dorsal aspect of the embryonic disc. The primitive streak results from the proliferation and movement of cells of the epiblast to the median plane of the embryonic disc. As the streak elongates by addition of cells to its caudal end, its cranial end proliferates to form a primitive node. Concurrently, the primitive groove develops in the primitive streak, and is continuous with a small depression in the primitive node - the primitive pit.

Shortly after the primitive streak appears, cells leave its deep surface and form mesenchyme, a tissue consisting of loosely arranged cells suspended in a gelatinous matrix. Mesenchymal cells are ameboid and actively phagocytic. Mesenchyme forms the supporting tissues of the embryo, such as most of the connective tissues of the body and the connective tissue framework of glands. Some mesenchyme forms mesoblast (undifferentiated mesoderm), which forms the intraembryonic, or embryonic, mesoderm. Cells from the epiblast as well as from the primitive node and other parts of the primitive streak form the embryonic endoderm in the roof of the umbilical vesicle. The cells remaining in the epiblast form the embryonic ectoderm. Mesenchymal cells derived from the primitive streak migrate widely. These pluripotential cells have the potential to proliferate and differentiate into diverse types of cells, such as fibroblasts, chondroblasts, and osteoblasts. In summary, cells of the epiblast, through the process of gastrulation, give rise to all three germ layers in the embryo, the primordia of all its tissues and organs.

**Early Stages Of Limb Development**

Limb development begins with the activation of a group of mesenchymal cells in the lateral mesoderm. The limb buds form deep to a thick band of ectoderm. Toward the end of the fourth week, the limb buds first appear as elevations of the ventrolateral body wall. The upper limb buds are visible by day 26 or 27, and the lower limb buds appear 1 or 2 days later. Each limb bud consists of a mass of
mesenchyme covered by ectoderm. The mesenchyme is derived from the somatic layer of lateral mesoderm.

![Diagram of embryonic development](image)

The limb buds elongate by proliferation of the mesenchyme. The upper limb buds appear disproportionately low on the embryo's trunk because of the early development of the cranial half of the embryo. The earliest stages of limb development are alike for the upper and lower limbs. Because of their form and function, there are many distinct differences between the development of the hand and foot. The upper limb buds develop opposite the caudal cervical segments, and the lower limb buds form opposite the lumbar and upper sacral segments.

At the apex of each limb bud, the ectoderm thickens to form an apical ectodermal ridge (AER). The AER, a multilayered epithelial structure, is induced by the underlying mesenchyme. The AER exerts an influence on the limb mesenchyme that initiates growth and development of the limbs in a proximo-distal axis. Experimental studies show that expression of endogenous fibroblast growth factors in the AER are essential for this process. Mesenchymal cells aggregate at the posterior margin of the limb bud to form the zone of polarizing activity. Fibroblast growth factors from the AER activate the zone of polarizing activity, which causes expression of the sonic hedgehog (Shh) genes. It has been suggested that Shh secretions (morphogens) control the patterning of the limb along the anterior-posterior axis.
The mesenchyme adjacent to the AER consists of undifferentiated, rapidly proliferating cells, whereas mesenchymal cells proximal to it differentiate into blood vessels and cartilage bone models. The distal ends of the limb buds flatten into paddle-like hand- and footplates. Laboratory studies have shown that endogenous retinoic acid is also involved in limb development and pattern formation.

By the end of the sixth week, mesenchymal tissue in the handplates has condensed to form digital rays. These mesenchymal condensations outline the pattern of the digits or fingers. During the seventh week, similar condensations of mesenchyme form digital rays and toes in the footplates. At the tip of each digital ray, a part of the AER induces development of the mesenchyme into the mesenchymal primordia of the bones (phalanges) in the digits.

The spaces between the digital rays are occupied by loose mesenchyme. Soon the intervening regions of mesenchyme break down, forming notches between the digital rays. As the tissue breakdown progresses, separate digits (fingers and toes) are formed by the end of the eighth week. Programmed cell death (apoptosis) is responsible for the tissue breakdown in the interdigital regions, and it is probably mediated by bone morphogenetic proteins, which are part of the transforming growth factor β superfamily. Blocking these cellular and molecular events could account for syndactyly or webbing of the fingers or toes.
Final Stages Of Limb Development

As the limbs elongate, mesenchymal models of the bones are formed by cellular aggregations. Chondrification centres appear in the fifth week. By the end of the sixth week, the entire limb skeleton is cartilaginous.

Osteogenesis of long bones begins in the seventh week from primary ossification centres in the middle of the cartilaginous models of the long bones. Ossification centres are present in all long bones by the 12th week, although ossification of the foot bones is not complete until approximately 20 years of age.

From the dermomyotome regions of the somites, myogenic precursor cells also migrate into the limb buds and later differentiate into myoblasts, precursors of muscle cells. As the long bones form, the myoblasts aggregate and form a large muscle mass in each limb bud. In general, this muscle mass separates into dorsal (extensor) and ventral (flexor) components. The mesenchyme in the limb bud also gives rise to ligaments and blood vessels. The cervical and lumbosacral myotomes contribute to the muscles of the pectoral and pelvic girdles, respectively.
Early in the seventh week, the limbs extend ventrally. Originally the flexor aspect of the limbs is ventral and the extensor aspect dorsal, and the preaxial and postaxial borders are cranial and caudal, respectively. The developing upper and lower limbs rotate in opposite directions and to different degrees:

- The upper limbs rotate laterally through 90 degrees on their longitudinal axes; the elbows come to point dorsally and the extensor muscles lie on the lateral and posterior aspects of the limb.
- The lower limbs rotate medially through almost 90 degrees; the knees come to face ventrally and the extensor muscles lie on the anterior aspect of the lower limb.

Developmentally, the radius and the tibia are homologous bones, as are the ulna and fibula, just as the thumb and great toe are homologous digits. Synovial joints appear at the beginning of the fetal period, coinciding with functional differentiation of the limb muscles and their innervation.

**KEY LEARNING POINTS.**

1. Limb buds begin to appear towards the end of the fourth week.
2. Digital rays begin to appear in the foot during week seven.
3. Separation of the digits occurs by week 8.
4. Ossification centres are present in all long bones by week twelve.
5. The lower limbs rotate medially through approximately 90 degrees.
Cutaneous Innervation of Limbs

There is a strong relationship between the growth and rotation of the limbs and their cutaneous segmental nerve supply. Motor axons arising from the spinal cord enter the limb buds (fifth week), growing into the dorsal and ventral muscle masses. Sensory axons enter after the motor axons and use them for guidance. Neural crest cells, the precursors of Schwann cells, surround the motor and sensory nerve fibres in the limbs and form the neurilemmal and myelin sheaths.

During week five, peripheral nerves grow from the developing limb plexuses (brachial and lumbosacral) into the mesenchyme of the limb. The spinal nerves are distributed in segmental bands, supplying both dorsal and ventral surfaces of the limb. A dermatome is the area of skin supplied by a single spinal nerve and its spinal ganglion; however, cutaneous nerve areas and dermatomes show considerable overlapping. With elongation, the cutaneous distribution of the spinal nerves migrates along the limbs, not reaching the surface in the distal part of the limbs. Although the original dermatomal pattern changes during growth of the limbs, an orderly sequence of distribution can still be recognized in the adult.

A cutaneous nerve area is the area of skin supplied by a peripheral nerve. If the dorsal root supplying the area is cut, the dermatomal patterns indicate that there may be a slight deficit in the area indicated. Because there is overlapping of dermatomes, a particular area of skin is not exclusively innervated by a single segmental nerve. The limb dermatomes may be traced progressively down the lateral aspect of the upper limb and back up its medial aspect. A comparable distribution of dermatomes occurs in the lower limbs, which may be traced down the ventral aspect and then up the dorsal aspect of the lower limbs. When the limbs descend, they carry their nerves with them; this explains the oblique course of the nerves arising from the brachial and lumbosacral plexuses.
Blood Supply to the Limbs

The limb buds are supplied by branches of the intersegmental arteries which arise from the aorta and form a fine capillary network throughout the mesenchyme. The primordial vascular pattern consists of a primary axial artery and its branches, which drain into a peripheral marginal sinus. Blood in the marginal sinus drains into a peripheral vein. The vascular patterns change as the limbs develop, chiefly by angiogenesis (sprouting from existing vessels). The new vessels coalesce with other sprouts to form new vessels. The primary axial artery becomes the brachial artery in the arm and the common interosseous artery in the forearm, which has anterior and posterior interosseous branches. The ulnar and radial arteries are terminal branches of the brachial artery. As the digits (fingers) form, the marginal sinus breaks up and the final venous pattern, represented by the basilic and cephalic veins and their tributaries, develops. In the thigh, the primary axial artery is represented by the deep artery of the thigh (profundus femoris artery). In the leg, the primary axial artery is represented by the anterior and posterior tibial arteries.
**Development of nails.**

At around 10 weeks finger and toenails begin to appear at the ends of the digits. These begin as small, thickened areas, known as nail fields, situated right at the tip of the digit. These areas begin to move proximally, over the dorsal surface of the digit, but as they were originally innervated from the plantar / palmar aspect, this innervation also follows the nail field, hence the importance of affecting the plantar digital nerves when performing a digital block with local analgesia.

Around the nail fields are the nail folds, and it is from the proximal nail fold that cells begin to divide and spread over the nail field. These cells keratinised to form the nail plate. Initially, the new nail plate is covered by a thin epithelial layer known as the eponychium, but over time this recedes, and remains at the base of the nail only.

By the end of the thirty-second week the fingernails should have reached the tips of the digits, but this is slower in the toenails, which take around thirty-six weeks. If the nails have not reached the end of the digits at birth, this is a sign of prematurity. Absence of nails at birth is very rare, and is usually associated with absence of hair and developmental abnormalities of teeth.
KEY LEARNING POINTS.

1. Motor axons arising form the spinal cord enter the limb bud during week five.

2. Sensory axons follow motor axons, and use their position for guidance.

3. A dermatome is the area of skin supplied by a single spinal nerve.

4. Cutaneous distribution relates to an area supplied by a peripheral nerve.

5. There is considerable overlap between dermatomes and cutaneous distributions.

6. As the limb increases in length, the nerves are carried with them.

7. Intersegmental arteries arise from the aorta and supply the limb buds.

8. The primal axial artery is the first vessel to supply the developing limb.

9. Angiogenesis is necessary for new blood vessels to develop.

10. The primal axial artery in the lower limb becomes the anterior and posterior tibial arteries.
Section 2 : Anomalies Of The Limbs

Learning Objectives.

At the end of this section, you should be able to :

1. understand how limb defects may occur during the pre-natal period
2. identify the more common congenital abnormalities affecting the lower limb
3. appreciate how these abnormalities may present, and forecast the types of problems likely to be encountered in later life relating to these deformities.

Minor limb anomalies are relatively common and can usually be corrected surgically. Although these anomalies are usually of no serious medical consequence, they may serve as indicators of more serious anomalies and may be part of a recognizable pattern of birth defects.

The most critical period of limb development is from 24 to 36 days after fertilization. This statement is based on clinical studies of infants exposed to thalidomide, a potent human teratogen, during the embryonic period. Exposure to this teratogen before day 33 may cause severe limb defects, such as amelia, the absence of limbs. Consequently, a teratogen that could cause amelia of the limbs or parts of them must act before 36 days, the end of the critical period of limb development. Many severe limb anomalies occurred from 1957 to 1962 as a result of maternal ingestion of thalidomide. This drug, widely used as a sedative and antinauseant, was withdrawn from the market in December 1961. Since that time, similar limb anomalies have rarely been observed. Because thalidomide is now used for the treatment of leprosy and several other disorders, it must be emphasized that thalidomide is absolutely contraindicated in women of child-bearing age.

Major limb anomalies appear in 2 in 1000 births. Most of these defects are caused by genetic factors. Molecular studies have implicated gene mutations in some cases of limb defects. Several unrelated congenital anomalies of the lower limb were found to be associated with a similar aberrant arterial pattern, which might be of some importance in the pathogenesis of these defects.
Limb Anomalies

There are two main types of limb anomalies:

- Amelia, absence of a limb
- Meromelia (Greek, *meros*, part, and *melos*, limb), absence of part of a limb.

Anomalies or defects of the limbs originate at different stages of development. Suppression of limb bud development during the early part of the fourth week results in absence of the limbs, amelia. Arrest or disturbance of differentiation or growth of the limbs during the fifth week results in various types of meromelia. Like other congenital anomalies, limb defects are caused by:

- Genetic factors, e.g., chromosomal abnormalities
- Mutant genes as in brachydactyly or osteogenesis imperfecta, a severe limb defect with fractures occurring before birth
- Environmental factors, e.g., teratogens such as thalidomide
- A combination of genetic and environmental factors (multifactorial inheritance), e.g., congenital dislocation of the hip
- Vascular disruption and ischemia, e.g., limb reduction defects

Experimental studies support the suggestion that mechanical influences during intrauterine development may cause some limb defects. A reduced quantity of amniotic fluid (oligohydramnios) is commonly associated with limb deformations; however, the significance of *in utero* mechanical influences on congenital postural deformation is still open to question.

Cleft Hand and Cleft Foot

In lobster-claw deformities, there is absence of one or more central digits, resulting from failure of development of one or more digital rays. The hand or foot is divided into two parts that oppose each other like lobster claws.
**Brachydactyly**

Shortness of the digits (fingers or toes) is the result of reduction in the length of the phalanges. This anomaly is usually inherited as a dominant trait and is often associated with shortness of stature.

**Polydactyly**

The term supernumerary digits refers to the presence of more than the usual number of fingers or toes. Often the extra digit is incompletely formed and lacks normal muscular development. If the hand is affected, the extra digit is most commonly medial or lateral rather than central. In the foot, the extra toe is usually on the lateral side. Polydactyly is inherited as a dominant trait.

**Syndactyly**

Syndactyly is the most common anomaly of the hand or foot. Cutaneous syndactyly (simple webbing between digits) is the most common limb anomaly. It is more frequent in the foot than in the hand. Cutaneous syndactyly results from failure of the webs to degenerate between two or more digits. Osseous syndactyly (fusion of the bones-synostosis) occurs when the notches between the digital rays fail to develop; as a result, separation of the digits does not occur. Syndactyly is most frequently observed between the third and fourth fingers and between the second and third toes. It is inherited as a simple dominant or simple recessive trait.
Congenital Clubfoot
Any deformity of the foot involving the talus is called talipes, or clubfoot. Talipes equinovarus is the most common type. Clubfoot is a relatively common anomaly, occurring approximately 1 in 1000 births. It is characterized by an abnormal position of the foot that prevents normal weight bearing. The plantar aspect of the foot is turned medially and the foot is inverted. Clubfoot is bilateral in approximately 50% of cases, and it occurs approximately twice as frequently in males. The cause of clubfoot is uncertain. Although it is commonly stated that clubfoot results from abnormal positioning or restricted movement of the fetus's lower limbs \textit{in utero}, the evidence of this deformation is inconclusive. Clubfoot appears to follow a multifactorial pattern of inheritance; hence, any intrauterine position that results in abnormal positioning of the feet may cause clubfeet if the fetus is has a genetic predisposition to this deformity.
Congenital Dislocation of the Hip
This deformity occurs approximately 1 in 1500 newborn infants and is more common in females than in males. The joint capsule is very relaxed at birth, and there is underdevelopment of the acetabulum of the pelvis and the head of femur. Dislocation almost always occurs after birth. There are two causative factors:

- Abnormal development of the acetabulum occurs in approximately 15% of infants with congenital dislocation of the hip, which is common after breech deliveries, suggesting that breech posture during the terminal months of pregnancy may result in abnormal development of the acetabulum and the head of femur.
- Generalized joint laxity is often a dominantly inherited condition that appears to be associated with congenital dislocation of the hip, which follows a multifactorial pattern of inheritance.

Note un-equal alignment of patella in both images.
Summary Of Limb Development

- Limb buds appear toward the end of the fourth week as slight elevations of the ventro-lateral body wall. The upper limb buds develop approximately 2 days before the lower limb buds. The tissues of the limb buds are derived from two main sources: mesoderm and ectoderm.
- The AER exerts an inductive influence on the limb mesenchyme, promoting growth and development of the limbs. The limb buds elongate by proliferation of the mesenchyme within them. Apoptosis (programmed cell death) is an important mechanism in limb development, for example, in the formation of the notches between the digital rays.
- Limb muscles are derived from mesenchyme (myogenic precursor cells) originating in the somites. The muscle-forming cells (myoblasts) form dorsal and ventral muscle masses. Nerves grow into the limb buds after the muscle masses have formed. Most blood vessels of the limb buds arise as buds from the intersegmental arteries and drain into the cardinal veins.
- Initially, the developing limbs are directed caudally; later they project ventrally, and finally, they rotate on their longitudinal axes. The upper and lower limbs rotate in opposite directions and to different degrees.

The majority of limb anomalies are caused by genetic factors; however, many limb defects probably result from an interaction of genetic and environmental factors (multifactorial inheritance).