Fertilization details:

Fertilization begins with gamete fusion (zygote formation). The fusion of a spermatozoon with a secondary oocyte takes place in the uterine tube, near the ovary:

- to begin, a spermatozoon binds to a specific glycoprotein on the zona pellucida that surrounds the oocyte [this recognition process precludes union with foreign sperm];
- the spermatozoon releases degradative enzymes (acrosomal reaction) that allows the sperm cell to penetrate the zona pellucida;
- spermatozoon and oocyte plasma membranes fuse (the secondary oocyte completes meiosis);
- the oocyte precludes fusion with other sperm by immediately canceling its membrane potential (via Ca⁺⁺ influx) and then by denaturing its zona pellucida (via enzymes released by exocytosis from oocyte cytoplasmic granules);
- male & female haploid pronuclei make contact, lose their nuclear membranes, and begin mitosis (mitosis begins 12 hours after sperm fusion; DNA synthesis takes place before mitosis)

Fertilization ends with the initiation of zygote cell division (the start of cleavage)

Cleavage:

This term refers to the series of mitotic divisions by which the large zygote is fractionated into numerous "normal size" cells. Each daughter cell of the cleavage process is termed a *blastomere*.

- cleavage begins with a *zygote*, progresses through compaction to a *morula* stage and terminates at the start of the *blastocyst* (blastula) stage
- the first eight blastomeres are undifferentiated and have identical potential in domestic mammals; thereafter, blastomeres differentiate into inner & outer cells with different missions

Note: The first cleavage division occurs 1 to 5 days following ovulation (depending on species), thereafter cells divide about once every 12 hours;

As many as eight generations of mitoses may occur without intervening cell growth (cytoplasmic increase). Thus, e.g., one 150 micron diameter zygote can becomes a collection of 256 cells, each about 7 microns in diameter.



A **morula** [L.= small mulberry] is a solid ball of blastomeres, within a zona pellucida. A morula typically consists of 16 to 64 blastomeres = four to six cell divisions. Blastomeres become compacted; cells packed on the inside differentiate from those along the surface of the morula:

outer blastomeres become flattened and form tight junctions (resulting in reduced permeability to fluids); they develop the capacity to secrete fluid (internally); they are destined to become *trophoblasts* which form the chorion & amnion (fetal membranes);

inner blastomeres form gap junctions to maximize intercellular communication; they are destined to become *inner cell mass* which forms the embryo (plus two fetal membranes).

Gastrulation:

Gastrulation is the morphogenic process that gives rise to three germ layers: ectoderm, mesoderm, and endoderm. (In a *gastrula* [Gr.= little stomach] one can see evidence of primitive gut formation.) Gastrulation includes the following sequence, beginning with a blastocyst:

- A thickened embryonic disc becomes evident at the blastocyst surface, due to cell proliferation of the inner cell mass cells. Trophoblast cells overlaying the inner cell mass degenerate in domestic mammals (in some mammals, e.g., mouse and human, trophoblast cells overlaying the inner cell mass separate and, instead of degenerating, become amnionic wall.)
- From the inner cell mass, cells proliferate, break loose (delaminate), and migrate to form a new cell layer inside the trophoblast layer. The new layer of cells is called the *hypoblast*; it forms a yolk sac. The remaining inner cell mass may henceforth be called *epiblast*.
- On the epiblast surface, a **primitive streak** forms as differential cell growth generates a pair of ridges separated by a depression. [NOTE: The primitive streak defines the longitudinal axis of the embryo and indicates the start of germ layer formation.]
- The separation of the hypoblast layer from the epiblast establishes a space (coelom/celom) deep to the primitive streak. Subsequently, the coelom is temporarily filled by mesoderm that undergoes cavitation to restablish the coelom that gives rise to body cavities.
- Epiblast cell proliferation along primitive streak ridges becomes the source of a cellular migration through the streak depression. The migrating cells form endoderm & mesoderm layers.





- Initial migrating cells join the hypoblast layer, forming embryonic **endoderm.** (The hypoblast constitutes yolk sac endoderm.)
- The majority of migrating cells enter the coelom as primary mesenchyme and become mesoderm. The primary mesenchyme migrates laterally and cranially (but not along the midline region directly cranial to the primitive streak where notochord will form). Note: Mesoderm divides into: paraxial, intermediate, and lateral mesodermal regions.



- Cavitation re-establishes a coelom (hoseshoe-shaped) within the lateral mesoderm. The mesoderm splits into two layers bordering the coelom—*somatic mesoderm* is attached to the ectoderm and *splanchnic mesoderm* is joined to endoderm.
- The remaining epiblast becomes ectoderm which forms skin epidermis & nervous system.



Formation of the notochord:

The **notochord** is a rod-shaped aggregate of cells located cranial to the primitive streak of the embryo. It occupies the midline coelomic space between ectoderm and endoderm that was not invaded by migrating primary mesenchyme.

The notochord is important because it induces formation of the head, nervous system development, and somite formation. It marks the future location of the vertebral column and the base of the cranium. Its ultimate fate is to become the nucleus pulposus of intervertebral discs.

The notochord develops from the *primitive node* located at the cranial end of the primitive streak. From the node, mesoderm-forming cells proliferate and migrate forward into the future head region where they become the rod-shaped notochord.

Longitudinal Section Through Primitive Node and Notochord







Early Formation of the Nervous System (Neurulation):

Neurulation refers to notochord-induced transformation of ectoderm into nervous tissue. The process begins during the third week in the region of the future brain and then progresses caudally into the region of the future spinal cord.

The following steps are involved in neurulation:

- ectodermal cells overlaying the notochord become tall columnar (neuroectoderm); they form a thickened area designated the *neural plate*. Other ectodermal epithelium is flattened.
- a neural groove is formed as edges of the neural plate become raised on each side of a midline depression. (Apical ends of individual neuroectodermal cells constrict.)
- a neural tube then forms as the neural groove undergoes midline merger of its dorsal edges. The tube separates from non-neural ectoderm which unites dorsal to it. (Tube formation begins in the cranial cervical region of the central nervous system



Neurulation

and progresses cranially and caudally until anterior and posterior neuropores, the last openings, finally close.)

 bilaterally, where the neural groove is joined to non-neural ectoderm, cells detach as the neural groove closes; the cells proliferate and assume a position dorsolateral to the neural tube—forming *neural crest*.

NOTE:

Neural tube becomes the central nervous system, i.e., the brain and spinal cord.

<u>Neural crest</u> cells are remarkable for the range of structures they form. Some cells migrate dorsally and become pigment cells in skin. Other cells migrate ventrally and become neurons and glial cells of the peripheral nervous system, or adrenal medulla cells. In the head, neural crest forms mesenchyme (ectomesenchyme) which becomes meninges, bone, fascia, and teeth.

Note: Each organ system has a critical period during development when it is most sensitive to external agents (teratogens) that produce birth defects.















Somite formation:

Somites are blocks of mesoderm located just lateral to the notochord. Generally, there are a pair of somites for every vertebra and a half dozen somite pairs in the head. The number of somites in an embryo is indicative of age because somites develop chronologically, in craniocaudal order.

Note: The ventromedial portion of a somite develops into a *sclerotome* (which forms vertebrae, ribs, & basal bones of the skull), the lateral portion becomes a *dermatome* (skin dermis), and the rest of the somite forms a *myotome* (skeletal muscle).

Somites develop as follows:

- mesoderm accumulates on each side of the notochord; this medially positioned mesoderm is designated *paraxial mesoderm*
- progressing from rostral to caudal over time, transverse fissures divide the paraxial mesoderm into blocks
- each block is a somite (epithelioid cells within a somite block re-orient 90°, from transverse to the notochord to longitudinal)
- head (occipital) somites develop



from proliferation of local mesenchyme lateral to the cranial end of the notochord

 rostral to the notochord, mesenchyme forms less-developed somites, called *somitomeres*, which migrate into pharyngeal arches and form muscles of the jaw, face, pharynx, & larynx.

NOTE:

Mesoderm can exist in two morphologic forms: mesenchyme and epithelioid: **Mesenchyme** features aggregates of stellate cells within an abundant extracellular matrix composed of fluid and macromolecules (polymers).

Epithelioid refers to organized cells having distinct apical and basal surfaces; the latter commonly rests on a basal lamina produced by epithelioid secretion.

- Mesoderm can transform from a mesenchyme to epithelioid and vice versa: The mesoderm that streams through the primitive streak is *primary mesenchyme*. Somatic, splanchnic, and somite mesoderm can be temporarily epithelioid. The temporary epithelioid transforms to a *secondary mesenchyme* which ultimately forms muscle and connective tissue (including cartilage, bone, ligaments, tendons, dermis, fascia, and adipose tissue).
- Thus, the term "mesenchyme" refers to the morphologic appearance of embryonic tissue. Although most mesenchyme is mesoderm, the other germ layers can also form mesenchyme, e.g., ectomesenchyme from neural crest ectoderm.





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Development of a Cylindrical Body:

The early embryo is flat, but the vertebrate body plan features a cylindrical theme—various cylindrical structures (derivatives of the gut, neural tube, notochord, etc.) enclosed within a cylindrical body. Transition from a flat embryo to a cylindrical one involves the following developments:

Head Process Formation:

• The cranial end of the embryo grows dorsally and forward so that it projects above the region originally in front of the embryo.

• The cylindrical head process elongates by growth from its base, located in front of the primitive node. Consequently, the most anterior part of the embryo is the oldest. The elongation incorporates the most anterior half-dozen somites into the future head.

• Within the head process, endoderm is reflected ventrally upon itself, forming a blind-ended *foregut* (future pharynx).

Tail Fold Formation:

• At the caudal end of the embryo, a cylindrical tail fold is formed in a manner similar to that of the head process.

• Folded endoderm encloses a blind hindgut .

Lateral Body Folds:

• As the head process elongates upward & forward, a subcephalic pocket (space) is formed ventral to the head process, between the head process and extra-embryonic tissue. The bilateral margins of this pocket are lateral body folds—which constitute the continuity between the elevated embryo and the relatively flat extra-



embryonic tissue.

• Similar folds exist caudally in association with the tail process.

• As the embryo

grows and is elevated dorsally, lateral body folds adduct and join together ventrally, establishing a tubular embryo separated from flattened extra-embryonic tissue.

• Progressing caudally from the head process and cranially from the tail fold, ventral fusion of lateral body folds stops at the *umbilicus*—leaving a ventral opening in the body wall that allows vessels and the yolk sac and allantois to enter the embryo (and communicate with the gut).

Three Stages of Head Process Formation (longitudinal views)











• Ventral fusion of lateral body folds distinguishes the embryo from extra-embryonic tissue (fetal membranes):

Embryonic coelom (future body cavities of the trunk) is distinguished from extraembryonic coelom within fetal membranes.

Somatopleure (somatic mesoderm + ectoderm) that forms body wall is distinguished from that forming fetal membranes (chorion and amnion).

Splanchnopleure (splanchnic mesoderm + endoderm) merges bilaterally to form gut and mesentery, differentiated from extraembryonic yolk sac (and allantois).



Pharyngeal (Branchial) Arches:

In the head region, anterior to the embryonic coelom, mesenchyme between ectoderm and endoderm forms a series of dorso-ventral arches demarcated by grooves (clefts). Only the first three *pharyngeal arches* are externally evident in mammals. Each arch contains a vessel (aortic arch). Within each arch, ectomesenchyme (derived from neural crest) gives rise to bone and fascia. Myotomes of somitomeres migrate to pharyngeal arches to provide skeletal muslculature. Each arch is innervated by one cranial nerve.

The first pharyngeal arch develops into upper and lower jaws and muscles of mastication. The second into hyoid bones and muscles of the face. The remaining pharyngeal arches form hyoid bones, larynx and associated muscles. Each arch is innervated by a particular cranial nerve.

The pharynx (foregut) develops five bilateral diverticula that internally demarcate the pharyngeal arches. These *pharyngeal pouches* develop into auditory tube, parathyroid glands, thymus, etc.

NOTE: In fish, five or six branchial [Gr. = gill] arches are well developed. Cells degenerate where branchial clefts and pharyngeal pouches meet so that the pharynx communicates with the outside (this occurs only temporarily between the first two arches in mammals). The first arch forms the jaw apparatus and the rest form gill arches separated by gill slits.

Flexures:

The tube-shaped embryo undergoes three flexures that make it C-shaped. The first occurs in the future midbrain region, the second in the future neck region, and the third occurs in the tail region.

Cardiovascular system:

• The cardiovascular system develops early (in the third week after the start of the nervous system), as the embryo enlarges and diffusion alone becomes inadequate for tissue preservation.

• Angiogenesis (formation of blood vessels) begins in splanchnic mesoderm of the yolk sac, in the form of blood islands composed of mesenchyme and hemocytoblasts. The latter forms blood cells and the mesenchyme forms vesicles lined by endothelium. The vesicles coalesce to form vascular channels and then blood vessels (the latter are formed by budding, fusion, & enlargement).

• Vessels are formed first in extra-embryonic tissue: vitelline (yolk sac) and umbilical (allantoic) vessels appear first.

• Ventral to the pharynx, bilateral vessels merge to form a tubular heart; dorsal and ventral aortae are connected by aortic arches. Also, cranial and caudal cardinal veins return embryonic blood to the heart and umbilical veins return placental blood to the heart. None of these vessels will persist as such in the adult.













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Placentation:

The placenta is the area(s) of apposition between uterine lining and fetal membranes where metabolites are exchanged for sustaining pregnancy.

Apposition areas (placental types) may be *diffuse* (pig), *zonary* (carnivore), *discoid* (primates & rodents), or involve placentomes. A *placentome* is a discrete area of interdigitation between a maternal caruncle and a fetal cotyledon. Equine placentas are microcotyledonary (microplacentomes are distributed diffusely). Ruminant placentas consist of rows of relatively large placentomes.

Placentas (placentae) may also be classified according to the tissue layers separating fetal and maternal blood. Uterine epithelium, uterine connective tissue and uterine endothelium may be eroded, giving rise to four placental types: epitheliochorial (swine, equine, cattle); synepitheliochorial, formerly called syndesmochorial, (sheep, goats); endothelial chorial (carnivore); and hemochorial (primates & rodents).



Fetal membranes:

Four fetal membranes develop in a conceptus. Two arise from the trophoblast layer of the blastocyst (and are continuous with the somatopleure of the embryo). Two arise from the inner cell mass of the blastocyst (and are continuous with splanchnopleure of the embryo); these two splanchnopleure membranes are vascular. The four fetal membranes are:

1. Chorion — from trophoblast, forms the outer boundary of the entire conceptus.

2. **Amnion** — from trophoblast, is formed by folds of chorion in domestic animals (in humans, amnion forms by caviation deep to a persistent trophoblast). The amnion encloses the embryo within a fluid-filled amnionic cavity.

3. Allantois — from the inner cell mass, develops as an outgrowth of hindgut splanchnopleure. The allantois grows to fill the entire extra-embryonic coelom, with fluid-filled allantoic cavity. The outer surface of allantois binds to the inner surface of chorion and the outer surface of amnion. The allantois is highly vascular and provides the functional vessels of the placenta, via umbilical vessels.

4. **Yolk sac** — from the inner cell mass, develops early (with hypoblast formation) and is continuous with midgut splanchnopleure. Supplied by vitelline vessels, yolk sac is most important in egg laying vertebrates. It forms an early temporary placenta in the horse and dog.

Note: The term *conceptus* refers to the embryo or fetus plus its fetal membranes.

Implantation

Following zona pellucida rupture, the blastocyst is innitially free in the uterine lumen (nourished by uterine glands). Implantation of the mobile blastocyct is a gradual process beginning with apposition leading to adhesion (or invasion in the case of the human & Guinea Pig). Approximate implantation times are: one week (human); two weeks (dog, cat, sheep), 3-5 weeks (cattle), 3-8 weeks horse; or delayed up to 4 mons (deer, bears).

Fetal Components of Placentae







Image From Placenta Courseware by Dr. V. Cox









