Early Embryogenesis

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Embryogenesis is the formation of body structures and organs in the developing embryo. It requires cell proliferation and differentiation which is regulated by gene expression. This means that only certain genes within the common genome are translated into proteins.

Definitions

- **Embryonic period**: Time between fertilisation and organogenesis including blastulation and gastrulation; embryos are usually not yet identifiable as member of a species
- **Fetal period**: controversial definition, often defined as time between organogenesis and term or, alternatively, advanced stages of pregnancy; fetus can be recognised as member of a species
- **Conceptus:** product of conception → everything that develops from the zygote including the embryo proper and the extra-embryonic membranes

Fertilisation

= union of a haploid oocyte and a haploid spermatozoon to produce a diploid zygote

Oocytes are released from the **ovary** into the **uterine tube** (= ovarian/ fallopian tube, oviduct) during **ovulation**. In most species, ovulation occurs spontaneously at the end of **oestrus**, the period of sexual receptivity. **Primary oocytes** need to undergo two **meiotic divisions**, one occurring just before ovulation and the other one just after fertilisation. This results in the final **haploid oocyte** providing half the genetic material for a future embryo. It is surrounded by the **zona pellucida**, a thick, transparent, non-cellular layer and a layer of cells, the **corona radiata**.

Depending on the species, **sperm** is deposited inside or immediately outside of the **cervix** (= the entrance to the uterus). It needs to travel up through the entire length of the uterine body and horns and into the uterine tube. The **fertile life span** of spermatozoa after being deposited inside the female varies greatly between species. In cattle, sperm cells survive about 1.5 to 2 days, while they survive 9 to 11 days in bitches. During their time in the female tract, sperm undergo changes to their molecular surface (=**capacitation**).

After the spermatozoon has travelled through the uterus and arrives in the uterine tube, the complex process of fertilisation begins. In brief, the first capacitated spermatozoon arriving at the oocyte breaks through the corona radiata and binds to the zona pellucida which induces progressive breakdown and fusion of the sperm's membranes referred to as **acrosomal reaction**. Enzymes released during this process and sperm motility allow the sperm to penetrate the **zona pellucida**, after which the zona changes to become impenetrable for other sperm (= **zona reaction**). The sperm and oocyte plasma membranes fuse allowing the sperm nucleus to enter the oocyte and enlarge due to DNA decondensation forming the **male pronucleus**. The cell containing both the male and female pronucleus is referred to as **ootid**. To form a **zygote,** the nuclear membranes of the two pronuclei fuse into a single diploid nucleus and the chromatin inside condenses into **chromosomes** that align themselves for the first **mitotic division**. This division gives rise to two identical daughter cells, the **blastomeres**.

Cleavage and hatching of the blastocyst

Cleavage refers to the initial mitotic divisions of the embryo inside the zona pellucida occurring 1 to 5 days after ovulation (species-dependent). Thereafter, cells divide about once every 12 hours. Due to the confinement inside the zona pellucida, the total size of the developing embryo does not change while the number of cells increases through a 2-cell, 4-cell, 8-cell and 16-cell embryo. After this stage, the embryo is referred to as a **morula** (*latin:* morus = mulberry) and leaves the uterine tube

(=oviduct) to enter into the uterus. In the morula, two distinct cell populations form – the compacted outer **trophoblast/ trophectoderm** cells, which will eventually give rise to the **chorion** (fetal part of the placenta) and the **inner cell mass (ICM)**, which will give rise to the embryo proper. The trophoblast cells form tight junctions and begin to pump sodium into the intercellular space. Water follows osmotically forming the fluid-filled **blastocoele.** At this stage, the embryo is called **early blastocyst**. As the cells keep undergoing mitosis and more and more fluid enters the blastocoele, the **pressure** inside the zona pellucida increases. In addition, trophoblast cells start producing **proteolytic enzymes** that weaken the zona pellucida. In combination with **contractions** of the blastocyst sending **pressure pulses** through the zona pellucida, these factors finally cause the **zona pellucida to rupture** and the **blastocyst to hatch**. Once freed from the confines of the zona pellucida, the blastocyst undergoes **rapid growth**. In cows, for example, the embryo grows from 3 mm on day 13 post ovulation to 25 cm three days later and occupies both horns by day 18 post ovulation. In pigs, the blastocyst grows from 2 mm on day 10 to 200 mm 24 to 48 hours later (= 4-8 mm/ hour) and to 1 m by day 16. This growth is primarily due to the formation of the **extra-embryonic membranes**, which are formed prior to attachment to the uterus in most domestic species. In primates, the blastocyst implants in the uterus very soon after entry and the membranes form afterwards. In mares, the embryo remains spherical and does not grow as dramatically in the intial stages.

For **embryo transfer** in cattle, embryos are usually flushed between days 6 and 8 after ovulation and graded based on their developmental stage (ideally morula or early blastocyst) and quality. It is therefore important to be able to distinguish between the different stages.

Twins can develop from two separate ovulations, when each oocyte is fertilised by a separate spermatozoon during the same breeding cycle (**dizygotic/ fraternal twins**). Dizygotic twinning has a hereditary basis. Litter mates in species that routinely give birth to multiple offsprings at the same time also usually develop from separate ovulations. **Monozygotic (identical) twins** arise from a single oocyte fertilised by a singly spermatozoon. They can result from separation during the 2-cell stage leading to two separate blastocysts developing in the same zona pellucida. Alternatively, two separate ICM can develop in the same blastocyst or the ICM and trophoblast can split during the hatching of the blastocyst provided both newly formed embryos contain enough of both cell populations. Natural twinning occurs in 2-3% and 2-5% of all births in cows and sheep, respectively. In horses, twins have a very high prenatal mortality rate and twin pregnancies are usually reduced to a single pregnancy by veterinarians.

Gastrulation

Gastrulation refers to the process during which the **three primary germ layers (ectoderm, mesoderm** and **endoderm)** are formed. All organs in the adult animal can be traced back to these three layers which form through ordered cell migration.

Initially, the ICM cells proliferate forming the thickened **embryonic disc**. The trophoblast layer above this disc degenerates (except in primates and mice where it forms the amnionic wall). On the lower surface of this embryonic disc, cells proliferate and break loose (delaminate) forming the **primitive endoderm**, which is sometimes also referred to as **hypoblast** in line with avian terminology. It expands to form the inner lining of the blastocoele forming a bilaminar **yolk sac**. Along the midline of the remaining cells of the ICM (the **epiblast** portion), a pair of ridges, the **primitive streak,** form in midline starting from the caudal pole. Together with the **primitive node** on its cranial edge, the primitive streak forms the cranio-caudal axis of the developing embryo. The primitive streak works like a rip current funnelling newly produced cells into the fluid filled space between the two layers (**coelom**). Some of these cells make contact with the primitive endoderm/ hypoblast, replacing it and forming the **definitive endoderm**. The coelom is temporarily filled with **mesoderm** before being re-established within the mesoderm as body cavities. The **lateral mesoderm** elongates quickly away from the embryonic disc to help form the extraembryonic membranes in animals with a fast-growing blastocyst. The remaining epiblast becomes **ectoderm**. As the primitive streak regresses, the primitive node moves continuously caudal again, depositing cells beneath the ectoderm along the way. These cells form the **notochord**, which is essential for midline symmetry and neural tube formation and marks the future location of the vertebral column.

Extra-embryonic membranes

Placenta formation is essential to sustain a mammalian pregnancy. It is the area of apposition between the uterine epithelium and the fetal membranes, which can form diffusely throughout the entire uterus or only in discreet areas, depending on the species. It is responsible for the exchange of nutrients, oxygen and waste products between dam and fetus.

Formation of **the extra-embryonic membranes (=fetal membranes**) is an obligatory step to allow for this attachment. They are a set of four anatomically distinct membranes:

- The **yolk sac** is initially formed by the primitive endoderm surrounded by trophectoderm and later, as it regresses, it is surrounded by mesoderm. It is a transient extra-embryonic membrane in domestic animal species and regresses as the conceptus develops. Its cells contribute to gonad formation.
- The **chorion** is formed by the trophectoderm and the expanding mesoderm. As it develops, it pushes upwards in the region of the conceptus and begins to surround it with “wing-like” projections.
- The **amnion** is formed once these projections fuse over the dorsal portion of the embryo, hence it is also derived from a trophectoderm and a mesoderm layer. It is a fluid-filled structure that functions as both a protection from mechanical irritations and an anti-adhesive preventing the growing embryonic tissues from sticking together. It can be carefully palpated in the cow between days 30 and 45.
- The **allantois** forms as a small evagination from the hindgut collecting liquid waste from the embryo. It is surrounded by mesoderm and continues to expand and eventually fuses with the chorion to form the **allantochorion** or **chorioallantois**. This forms the fetal part of the placenta attaching the fetus to the maternal uterine epithelium. The allantois is highly vascular and contributes the umbilical vessels to the placenta.

Establishing the basic body plan in the embryo

Ectoderm development

Neurulation refers to the formation of nervous tissue from ectoderm. To induce this transformation, the notochord needs to send a signal to the overlying ectodermal cells to become tall columnar cells (**neuroectoderm**) forming a thickened area, the **neural plate**. On both sides, slight elevations, the **neural folds,** are present, while the plate becomes depressed in midline, forming the **neural groove**. The folds become higher until they eventually converge towards the dorsal midline and fuse together, forming the **neural tube** that is separate from the remaining ectoderm, now called **surface ectoderm**. Some neuroectodermal cells migrate from the developing neural tube and form the **neural crest**, situated dorso-lateral to the neural tube. The central nervous system (brain and spine) arises from the neural tube, while the peripheral nervous system develops from both the neural tube and crest. Neurulation starts at the cranial pole and slowly moves caudally, as the primitive streak regresses and the notochord forms.

The neural tube and crest cells induce some cells of the surface ectoderm in the head region to form discreet thickenings, the **nasal** (→ nasal chamber)**, lens** (→ eye) and **otic** (→ inner ear) **placodes.** **Neurogenic placodes** contribute to the sensory part of some cephalic nerves.

Mesoderm development

During neurulation, mesoderm adjacent to the neural tube forms a thickened column of cells, the **paraxial mesoderm**. These cells aggregate sequentially in a cranio-caudal direction (alongside neural tube formation) to form discrete blocks called **somites**. Most of the axial skeleton (vertebrae, ribs, basal bones of the skull) with associated muscles and the overlying dermis will arise from these somites. Rostral to the notochord, mesoderm whirls form 7 quasi-somites, the **somitomeres**. These migrate into pharyngeal arches and help form muscles of the jaw, face, pharynx and larynx.

Lateral to the paraxial mesoderm, the **intermediate mesoderm** forms, which will contribute to the urinary and reproductive system development.

The **lateral mesoderm** is divided into an outer **somatic** and an inner **splanchnic layer**. The somatic layer fuses with the ectoderm to form the **somatopleure**, while the splanchnic layer fuses with the endoderm forming the **splanchnopleure**. The space between the two layers is the intra-embryonic coelom (different to the primitive coelom during gastrulation!). It later forms the body cavities (pleural, pericardial and peritoneal cavities). The mesoderm lining these cavities is a simple squamous epithelium forming the serosa membranes (pleura, pericardium, peritoneum).

Endoderm development

The endoderm gives rise to the **epithelial lining** of the primitive gut, the respiratory tract, the bladder and parts of the auditory system and the parenchyma of the liver, pancreas, thyroid and parathyroid.

Embryonic folding

Embryonic folding refers to the process required to transform the flat embryonic disc into a 3-dimensional, tubular embryo. It involves several steps often happening simultaneously.

The cranial part of the neuroectoderm begins to grow above the surface ectoderm and endoderm layers creating the **head process** and the **subcephalic pocket** underneath. In the subcephalic pocket, the endoderm is attached to the surface ectoderm and thus gets elongated during this growth, forming the blind-ending, tube-like **foregut**.

At the caudal end, a similar process takes place forming the cylindrical **tail fold**. The endoderm also folds in a similar manner forming the blind **hindgut**. The embryo becomes **C-shaped**.

As the head process grows upwards, **lateral body folds** form to cover the sides of the process with surface ectoderm. They are continuous with the subcephalic pocket and separate the elevated head process from the flattened extra-embryonic membranes. As development proceeds and the embryo is elevated dorsally, lateral body folds progressively separate more and more of the embryo from the extra-embryonic membranes. An identical process starts at the tail end, progressively moving cranially until the two sets of body folds meet at the level of the **umbilicus**, the stalk connecting the embryo to its extra-embryonic structures. During this process, the embryonic coelom is distinguished from the extra-embryonic coelom surrounded by fetal membranes. In addition, the somatopleure and splanchnopleure are separated from the mesoderm lining of the extra-embryonic membranes.

The gut tube starts to develop at the same time neurulation occurs and mirrors the neural tube formation. The **left and right endodermal folds** grow downwards and fuse in the ventral midline, forming a closed endodermal tube. This process starts cranially and progressively moves caudally, later also proceeding cranially from the hindgut.

As the gut starts to close behind the hindbrain, **cardiogenic mesoderm** located beside the endodermal folds is pulled along to the ventral midline. **Endothelial tubes** form on each side before fusing to form a **single cardiac tube.** This cardiac tube will then expand, loop caudally and become subdivided into four chambers.

Pharyngeal arches, aortic arches and pharyngeal pouches

The pharynx develops a series of evaginations, the **pharyngeal pouches**, which separate the **neural crest cells** running dorsal into a series of **pharyngeal/ branchial arches**. Running through each pharyngeal arch is an **aortic arch** (connecting the ventral and dorsal embryonic aorta). The **first** of these arches separates and forms both the mandibular and the maxillary process. The **mandibular process** expands ventro-medially circling the pharynx and fusing together to form the lower jaw. The **maxillary process** expands underneath the eye. The second pharyngeal arch, the **hyoid arch**, also grows around the pharynx and fuses ventrally to form the hyoid bone, to which the third arch also contributes. Some facial muscles are also derived from the second and third pharyngeal arch. The fourth and sixth pharyngeal arches form the intrinsic muscles of the larynx, the constrictors of the pharynx and the cartilages of the larynx. Each arch is innervated by a particular cranial nerve. Pouch derivatives in the final body plan include the auditory tube, tonsils, and the thymus.

References and further reading

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