Cell Development

- Processes required for the development of a human being from a MORULA (ball of cells) include:
  1. Cell division/Proliferation
  2. Cell movement
  3. Differentiation
  4. Cell loss

Cell regulation

- Is determined by the location of receptors, not the location of initial secretion
  - Regulators are produced and secreted from a locus within a tissue; thereby exposing cells to a concentration gradient – closer cells will respond differently to cells that are further away from the source of production
- PARACRINE regulation: can be up or down/inhibitory - acts on adjacent cells
- AUTOCRINE regulation: common in embryology due to small cell size, regulators act on self function
- ENDOCRINE regulation: regulation between tissues via the bloodstream

Cell division/ Proliferation

- Cells closer to the source are exposed to an increase concentration of the stimulus, therefore grow more rapidly
- The resulting new growth of cells are further away from the initial source, therefore are exposed to a lower concentration of stimulus and grow more slowly
- This results in a base “LIMB BUD” structure of growth (standard lump shape)
- MODIFIED proliferation:
  - Modification by enhancers or inhibitors
  - Changes the shape of the growth

Cell movement

- In response to stimulants
- CHEMO-ATTRACTANTS: move cells towards their stimulus
- This requires remodelling of tissues (e.g. extra-cellular matrix proteins)

Differentiation

- Mostly controlled by paracrine factors
- Dependent on expression of specific receptors
- Differentiation results in a decrease in proliferation

Cell loss

- Apoptosis
- Regulated cell death
- Controlled by paracrine factors mainly
Embryonic development

Complex and coordinated repeated application of these 4 processes:

*Pre-implantation*

- Egg is fertilised at ovarian end of fallopian tube
- Over the next 4-5 days, the fertilised egg migrates down the fallopian tube towards the uterus
  - The egg also begins to proliferate/divide, increasing the number of cells
- The first sign of differentiation is at 5 days, with the formation of the BLASTOCYST
- On day 6/7, attachment of the blastocyst into the ENDOMETRIAL EPITHELIUM occurs

*After Implantation*

- Day 8-9: Implantation of the blastocyst and the formation of:
  - TROPHOBLAST SHELL
  - BILAMINAR EMBRYONIC DISC
- The bilaminar embryonic disc consists of:
  - EPIBLAST
  - HYPOBLAST
- The cavity above the epiblast is known as the AMNION
- The cavity below the hypoblast is known as the YOLK SAC
- The trophoblast shell consists of:
  - CYTOTROPHOBLAST
  - SYNCTIOTROPHOBLAST
- Day 12-13: trophoblast invasion and contact with MATERNAL BLOOD

*Developmental Timings*

- PREGNANCY is counted from last menstrual period
- DEVELOPMENT is counted from fertilisation
  - Blastocyst: 9 days
  - Embryo: 5-6 weeks
  - Fetus: 3 months

*How to make an embryo/fetus*

➤ **GASTRULATION and Germ Layer Formation**
- Day 15/16: the two layers of cells in the Bilaminar embryonic disc form a TRILAMINAR EMBRYONIC DISC, i.e. become three layers of cells. In reality, the epiblast forms the:
  - ECTODERM
  - MESODERM
- The mesoderm then further differentiates to form the ENDODERM
- The hypoblast is lost in this process
- This requires cell movement
- Fate of the germ layers:
  - Ectoderm → skin, nervous system
  - Mesoderm → skeleton, muscle, kidney, heart, blood
  - Endoderm → gut, liver, lungs
- **Notochord formation**
  - Differentiation of the embryonic disc occurs in parallel with gastrulation and germ layer formation
  - This results in the formation of the:
    - PRIMITIVE STREAK
    - PRIMITIVE NODE
    - The primitive streak sets up the ANTERO-POSTERIOR axis
  - Differentiation \(\rightarrow\) formation of the NOTOCHORD by day 17 from the primitive node
    - The notochord sets up the DORSAL-ANTRAL axis

- **Neurulation**
  - The NEURAL PLATE surrounds the notochord
    - This is a flat layer of CNS pre-cursor cells formed by the ectoderm germ layer
  - Day 19: NEURAL CRESTS also begin to form by the initial folding of the neural plate
    - This leads to the formation of the PARAXIAL and INTERMEDIATE MESODERM from the original mesoderm

- **Neural crest migration**
  - Day 20: migration of the neural crests occurs to form a NEURAL GROOVE
  - This leaves a much more definite:
    - PARIETAL MESODERM—surrounding the amnion
    - VISCERAL MESODERM—surrounding the yolk sac

- **Somitogenesis**
  - Day 21: fusion of the neural groove leads to the formation of the NEURAL TUBE
  - The intermediate mesoderm gives rise to the primary trunk of the embryo body, and SOMITES are formed

- **Folding and cavitation**
  - By day 21: increased proliferation gives rise to a more 3D structure as the tissue begins to fold
    - The amniotic cavity then begins to fold around the body wall (ectoderm)
    - The visceral mesoderm layer then begins to form the GUT WALL
    - The parietal mesoderm layer then begins to surround to INTRAEMBRYONIC CAVITY
  - Day 22/23: neural tube closes, and there is elaboration of structure at the head of the embryo:
    - Formation of the anterior and posterior NEUROPORE
    - PERICARDIAL BULGE forms
  - Day 25/18:
    - Heart bulge forms
- Limb ridges form
- Lens and otic placode forms
- Yolk sac begins forming UMBILICAL CORD
- Amniotic cavity completely surrounds embryo

Organogenesis
- 5th week -- Face & limbs continue. (5-8mm).
- 6th week -- Face, ears, hands, feet, liver, bladder, gut, pancreas. (10-14mm)
- 7th week -- Face, ears, fingers, toes. (17-22mm)
- 8th week -- Lungs, liver, kidneys, (28-30mm)

Genetic Regulation of Development
- Processes controlled by signals from genes
- Complex genetic interactions occur throughout development to form a normal fetus
- E.g. Hox Genes
  - Establish antero-posterior axis
  - Differences in the vertebrae
  - CNS divisions
  - Pattern limbs
    - One of the signals that controls Hox genes is Retinoic Acid (Vitamin A derivative)
- Gene signalling in different species is all quite similar, as many of the same genes found in both
- Nutritional make-up has an important influence on gene interactions

Patterning
- Pattern formation are the processes that convert the blastocyst from a bilaminar structure to an embryo with organs, specialised tissues, limbs and other body structures. This includes giving polarity to the embryo as well as laterality.
- Formation of specific structures during development of a tissue
- Controlled by expression patterns of regulators or receptors
- E.g. the AER (APICAL EXTODERMAL RIDGE) signals to underlying tissue to promote limb outgrowth
- E.g. 2. ZPA (ZONE of POLARIZING ACTIVITY) patterns the A/P axis
  - Sonic hedgehog is the polarizing factor of ZPA
    - Controls the differentiation of ZPA
Limb Development

- Forelimb bud appears at d27/28
- Hindlimb bud at d29
- Grow out from lateral plate mesoderm rapidly under control of special signalling regions
- Fully formed and patterned by d56.
Facial Development

- At 5 weeks, the eyes are on the side of the skull. In the next 5 weeks, movement of the eyes to the front of the skull occurs
- By 10 weeks, the nose has also formed
Embryological Malfunctions
HLC 2 - Dr Mark Sullivan (m.sullivan@imperial.ac.uk)

Overview

- Derangement of any of the 4 processes can lead to mal-development of an embryo, and hence delivered foetus. These processes include:
  - Proliferation
  - Cell movement – cells have to move at the right time and place to generate the correct tissues within a normal foetus
  - Differentiation
  - Cell loss – e.g. embryonic “tail” is loss during foetal development, and if this does not occur, the foetus would be delivered with a form of “tail” at the base of the spine
- As a result, the foetus may die in-utero; this may be spontaneous or clinically induced abortion after identification of malformation

Causes

- In early development, abnormalities are primarily due to chromosomal/genetic problems. The severity of the abnormality thus depends on the extent of chromosomal alteration
  - Only alterations in chromosomes 21, X and Y have full viability in humans
  - E.g. Down’s syndrome – trisomy 21 (however huge variation in severity of phenotype presentation)
  - No other chromosomal abnormalities are viable in foetuses which develop to full term, and survive after delivery
  - Any abnormalities in Chromosome 1 would not even survive to implantation into the uterus, as this is the largest chromosome thus carries the most genetic information – VITAL for embryonic development
  - Some chromosomal abnormalities will allow implantation of the blastocyst, but will lead to pregnancy loss, i.e. will not be viable in full term foetus
  - Alterations in chromosome 16 and 18 have been seen in full term delivery foetuses, but are not viable for human survival therefore the child will not survive
- During patterning events, abnormalities are due to the influence of genetic and/or environmental factors

Birth Defects

Two primary categories:
- **Physical**
  - Observable abnormality in foetal structure
  - Often due to mal-development during patterning events
- **Neurological**
  - Also developmental
  - Not easily observed
  - Become more apparent during post-delivery development
  - Range in severity, which also correlates to how apparent abnormality is
NB: not all birth defects are caused by pre-term mal-development, defects can be caused during delivery. This is more common in pre-term deliveries, which often lead to malformations.

**Spina Bifida**
- Neural tube defect- posterior neuropore doesn’t sloc
- Result- neural tissue is not constrained, therefore bulges out – this is seen by a bulge of neural tissue in the lower spine
  - Neural tissue also directs bone formation, therefore at the location of the bulge/lesion, there will be abnormal formation of the spine, seen by a hole
- Causes: multiple causes, e.g. Folate deficiency caused by inadequate folate intake in maternal diet during pregnancy

**Holt-Oram Syndrome**
- Seen by both heart and hand defects
- Atrial septation defects – gap between the ventricles, therefore mixing of oxygenated/deoxygenated blood occurs – impact of this depends on severity of gap
- Range of hand defects
  - Patterning events must be ALMOST normal, as 5 digits are formed
  - However, the thumb appears very similar to the 5th finger, and is not completely opposable
  - This is not a PRIMARY structural defect, but rather a SECONDARY structural defect whereby the detail is affected – hence there will be differences between the hands
- Phenotype cause by mutation in **TXB5 gene**
- Knowing the relationship of structure/organ embryonic development will help aid diagnosis

**Polydactyl**
- Duplication of a digit – 6 digits seen on hand
- Not a patterning defect, as primary structure fully formed
- Also leads to increased webbing between the extra and duplicated digit
- May also lead to other minor changes in structure of the hand, for example the orientation of the little finger – may be curved and slightly angled outwards

**Cleft lip & palate**
- Multiple causes, e.g. TBX22 (familial cause)
  - TBX22 causes movement of facial cells and fusion - but especially cells under nose
- Tissue underneath nose does not join together
- Fusion seen with other facial tissue, therefore other genes control facial tissue fusion – this is because it is a critical process within embryonic facial development

- Surgical treatment – repair tissues (especially effective in babies as heal rapidly)
- Cause: TBX22 gene not activated between weeks 5-10 of development
- Normal development: Nasal pits move closer together, and fusion occurs forming the PHILTRUM
  - Instead there is a persistent labial groove due to the failure of merging MEDIAL NASAL PROMINENCES
  - Also non-superficial tissues; PALATE, is controlled and affected by the malfunctioning TBX gene

**Achondroplasia**

- Form of dwarfism
- Caused by a lack of functioning FGFR3 (FGR receptor 3)
- Without receptor, the FGF cannot exert full effects on development, therefore development (and hence growth) does not occur to normal extent

**External Agents which cause birth defects**

- **Infectious agents**
  - Rubella virus - Cataracts, glaucoma, heart defects, deafness, teeth
  - Herpes simplex virus - Microphthalmia, microcephaly, retinal dysplasia
  - HIV - Microcephaly, growth restriction
  - Syphilis - Mental retardation, deafness

- **Physical agents**
  - X-rays - Microcephaly, spina bifida, cleft palate, limb defects

- **Chemical agents**
  - Thalidomide - Limb defects, heart malformations
  - Lithium - Heart malformations
  - Amphetamines - Cleft lip and palate, heart defects
  - Cocaine - Growth retardation, microcephaly, behavioral abnormalities
  - Alcohol - Fetal alcohol syndrome, maxillary hypoplasia, heart defects

NB: defects here develop primarily in the first trimester of pregnancy

**Teratogens**

- Definition: factor which can cause a birth defect
- These do not affect development in the first 2 weeks after blastocyst implantation, as there is no tissue formation.
  - Tissues are then differentially vulnerable to teratogens in the first weeks 3-7 of development
- Vulnerability of tissues with respect to weeks of development:
  - CNS: 3 - end of 7
  - Heart: 3.5 - 6.5
  - Upper limb: 4 - start of 7
  - Eyes: 4.5 - end of 7
  - Lower limb: 4.25 – 7.5
  - Palate: 6.75 – end of 7
  - Ear: 4.25 end of 7

*E.g. Thalidomide*

- Treatment for morning sickness
- Prevents upper limb (and sometimes lower limb) development
- Also targets vascular development, therefore deranges vasculature and blood supply especially to upper limbs
- However thalidomide has current interest with respect to its anti-inflammatory effects, and as a treatment for leprosy
  - However when used as a treatment for leprosy, still cannot be used during pregnancy

**Is sex necessary?**

HLC 3 - Professor John Laycock ([j.laycock@imperial.ac.uk](mailto:j.laycock@imperial.ac.uk))

- The key reason for sex is for procreation, i.e. to reproduce
- Also associated with pleasure – likely to involve specific neural pathways in the brain
- Responsibilities and behaviours associated with parenthood – linked to the activation of specific neuroendocrine pathways e.g. vasopressin

**The reproductive system**

In either sex, the reproductive system consists of:
- Gonads
- Internal duct system
- External genitalia

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<thead>
<tr>
<th>Reproductive System Component</th>
<th>Males</th>
<th>Females</th>
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</thead>
<tbody>
<tr>
<td>Gonads</td>
<td>Testes</td>
<td>Ovaries</td>
</tr>
<tr>
<td>Internal duct system</td>
<td>Seminiferous tubules</td>
<td>Uterine (fallopian) tubes – also known as the oviduct</td>
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<tr>
<td></td>
<td>Efferent ducts (testis)</td>
<td>Uterus</td>
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<td></td>
<td>Epididymis</td>
<td>vagina</td>
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<td>Vas deferens</td>
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<td></td>
<td>Urethra</td>
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<tr>
<td>External genitalia</td>
<td>Penis</td>
<td>vulva</td>
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<tr>
<td></td>
<td>Scrotum</td>
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**Testes**

- Housed in the scrotum
- Spermatic cord (vas deferens and associated structures e.g. prostate) leads to the back of the bladder and joins with seminal vesicle at ejaculatory duct
- Duct opens into urethra, which consists of prostatic, membranous and penile sections
Each testis consists of:
- Seminiferous tubules – involved in sperm production
- Sertoli cells – involved in sperm maturation process
- Interstitial leydig cells – production of testosterone

Ovaries
- Bulk is stroma – known as the cortex; contains follicles with oocytes
- Also vascular area; known as medulla
- No duct system like with testes
- At ovulation, developing “ovum” ruptures through ovarian wall (secondary oocyte)
- Follicles may be at different stages of development
- The secondary oocyte/ovum is picked up by end of fallopian tube and transported to the uterus
  - The ends of the fallopian tube has finger-like projections called fimbria which catch the ovum when it is released from the corona radiata within the ovary
- Vesicular (graffian) follicle
  - Liquor folliculi
  - First meiotic division – secondary oocytes (plus first ejected polar body) formed at ovulation
  - Second meiotic division – stops at metaphase, the oocyte is ovulated into the pelvic cavity where it is caught by fimbriae of the fallopian tube
    - The second meiotic division finishes after fertilisation

The menstrual cycle
- Changes take place within the 3 layers of the endometrium (uterine wall)
  - Stratum compactum - functional zone/layer in which vascular development and secretory changes take place
  - Stratum spongiosum - functional zone/layer in which vascular development and secretory changes take place
- **Stratum basale** – basal layer not shed during menstruation

- Cyclical event (~28 days long) in which changes take place in breasts, body temperature, endometrium etc

- Menstruation occurs if fertilization does not occur

- Consists of two cycles, both driven by the hypothalamus, with negative feedback loops:
  - **Ovarian cycle** – consists of 3 stages:
    - Follicular phase – secretion of oestrogen (17beta-oestradiol)
    - Ovulation – approx 2 day window for fertilization
    - Luteal phase – secretion of predominantly progesterone but also 17beta-oestradiol
  - **Endometrial cycle** – consists of 2 phases:
    - Proliferative phase – stimulated by the release of oestrogen from the ovarian cycle
    - Secretory phase – stimulated by the release of progesterone

- **Menstrual Phase:**
  - The first day of menstruation marks the beginning of the cycle
  - Last 4-5 days, flow of 20-80mls
  - Sheds the functional zone of the endometrial lining, along with blood
  - Remaining basal endometrium is very thin

- **Proliferative phase:**
  - Last ~9 days
  - Post-menstruation build up of endometrium
    - 2-3x increase in endometrial thickness; phase of repair and proliferation
    - Epithelium repairs
    - Gland number and length increases
    - Spiral arteries lengthen
  - Link with ovarian cycle –
    - Follicular phase \(\rightarrow\) growth of follicle
    - Stimulated by release of 17-beta oestradiol from ovaries
  - Hormonal control –
    - Hypothalamus – GRH
    - Pituitary gland – FSH and LH
    - Ovary – 17-beta oestradiol
- **Ovarian mid-cycle event: Ovulation**
  - LH surge vital for ovulation
  - Basal body temperature increase can be seen as an indicator

- **Secretory phase**
  - Production of nutrients and other secretory products
  - Last ~13 days
  - Link with ovarian cycle –
    - Occurs in response to 17-beta oestradiol and progesterone produced from corpus luteum during ovarian luteal phase
    - Corpus luteum formed after ovulation – release of secondary oocyte
  - Glandular epithelium – wide tortuous glands, progesterone secreting
  - Endometrium – thickening, progesterone and oestrogen secreting
  - Spiral arteries coil
Fertilization

- Menstrual cycles continue throughout a woman’s reproductive life, unless fertilization of a released ovum by a mature spermatozoon occurs → pregnancy
- Requires sperm and egg, and usually occurs following copulation (coitus) between a man and woman
  - Exception – IVF

Penis

- Vital as vector for sperm
- Tactile stimulus
  - Afferent fibres in pudendal nerve (somatic nervous system)
  - Spinal cord and brain (limbic system)
- Efferent fibres
  - Parasympathetic nervous system via pelvic nerve - promotes erection
  - Somatic nervous system via pudendal nerve – promotes erection
  - Sympathetic nervous system via hypogastric nerve - depression of erection
- Penis
- Sexual thoughts can also stimulate the same pathway
- The penile erection process involves changes from:
  - Flaccidity
  - Tumescence
  - Erection
- Erection is a vascular mechanism; it involves regulation of blood flow to the penis
- Within the penis, there are two different tissues, as well as the urethra:
  - Corpus spongiosum
  - Corpus cavernosum
- An increase in parasympathetic activity to the smooth muscle of the pudendal artery induces dilatation, which counteracts sympathetic-maintained myogenic tone and increases blood flow to the corpus cavernosum via the pudendal artery
  - The outflow of blood from the penis is reduced by compression of the dorsal vein following increase in corpus cavernosum pressure
  - The urethra is protected from this increased pressure by the surrounding corpus spongiosum, which is less turgid
- Mechanism of smooth muscle dilatation:
  - Activity in parasympathetic post-ganglionic fibres
  - Release of acetylcholine from the fibres into the smooth muscle cell
  - This stimulates nitric oxide synthase → release of NO, which activates the cyclic GMP pathway leading to dilation of arterial smooth muscle
  - Viagra inhibits the breakdown of cyclic GMP to 5’GMP, therefore leading to dilatation of the arterial smooth muscle
The clitoris

- Erectile tissue in women
- Increases in size as a result of increased blood flow into the tissue
- Same mechanism as erection process

Human Pregnancy in 1 hour
HLC 5 - Dr Mark Sullivan (m.sullivan@imperial.ac.uk)

Gametogenesis

- Before pregnancy, gametogenesis occurs in both males and females
- Male germ cell – spermatozoa
- Female germ cell – secondary oocyte
- Meiosis I leads to the haploid number of chromosomes in secondary oocytes and spermatocytes, ensuring no two offspring of parents are genetically identical
- Meiosis II of the secondary oocyte occurs after fertilization
Essential pregnancy

- **0-13 weeks: First Trimester**
  - Fertilisation
  - Implantation
  - Embryonic development
  - Maternal blood flow
  - NB: most dangerous trimester with regards to miscarriages

- **14-26 weeks: Second Trimester**
  - Fetal development
  - Change of development to growth
  - “limit of life”

- **27-39 weeks: Third Trimester**
  - Growth
  - Continuing development of brain, lungs, digestive system, immune system
  - Birth

### The First Trimester

**Fertilisation**

- Requires the acrosome reaction
- This occurs in the fallopian tubes at the distal end ampulla (the fimbrae of the uterine tube sweep the oocyte into the ampulla where it can be fertilized)
- The sperm penetrates the extraneous coats of the corona radiata and the zona pellucida - this prevents multiple sperm entry, nourishes developing conceptus and ensures implantation at appropriate site (i.e. prevents ectopic pregnancy)
- Next the plasma membrane of the sperm is stripped away and left behind as the sperm head and tail enter the oocyte.
- When the oocyte is contacted by the sperm, the oocyte completes its second meiotic division and as a result a mature oocyte and a second polar body is formed. The nucleus of the mature oocyte constitutes the female pronucleus
- After the sperm enters the oocyte, the head of the sperm separates from the tail and enlarges to become the male pronucleus.
• Fertilization is complete when the Pronuclei unite and the maternal and paternal chromosomes intermingle during metaphase of the first mitotic division of the zygote

**Post-fertilisation and Implantation**

• The zygote, as it passes along the uterine tube towards the uterus undergoes cleavage (a series of mitotic cell divisions) into a number of smaller cells called blastomeres.
• About three days later a ball of 12/16 blastomeres, called a morula, enters the uterus.
• A cavity soon forms in the morula converting it into a blastocyst. The blastocyst consists of:
  - the inner cell mass (or embryoblast)- which gives rise to the embryo
  - A blastocyst cavity- a fluid filled space
  - the trophoblast- a thin outer layer of cells which goes on the make the placenta (the Trophoblast encloses the inner cell mass and the blastocyst cavity).

• **Implantation:**
  - Four to five days after fertilization, the zona pellucida is shed and the Trophoblast adjacent to the inner cell mass attaches to the endometrium epithelium.
  - The inner cell mass delineate to give rise to a bilaminar disc:
    - the epiblast- which provides the amniotic cavity
    - the hypoblast- which forms the yolk sac
  - On the eighth day after fertilization, cells at the edge of the hypoblast migrate and cover the inner surface of the blastocyst wall. The migrating columnar cells become squamous and form a tiny membrane called the exocoelomic membrane. Together with the hypoblast, the exocoelomic membrane forms the wall of the yolk sac- formerly known as the blastocyst cavity. As a result the bilaminar disc is now positioned between the amniotic cavity in the epiblast and the yolk sac.

*Early embryonic development* - See other lecture notes

**Maternal blood flow**

• The placenta lies on the hyperproliferative glands of the endometrium
  - Trophoblast cells occlude the arteries of the endometrium, limiting the blood supply
• After 12 weeks of pregnancy, the placenta is exposed to the whole maternal blood supply, exposing the fetus to the full maternal blood pressure
  - If the placenta is not fully developed and anchored, this may lead to a miscarriage
• Past this stage, the risk of miscarriage is much less likely
Labour Stages

- There is a latent stage of the last ~8 weeks of pregnancy
  - During this stage, contraction is prevented
- Labour lasts 12-48 hours and consists of 3 phases:
  - Phase 1 – last many hours, period of contractions with cervical changes
  - Phase 2 – delivery of baby
  - Phase 3 – delivery of placenta

Intrauterine support of the embryo and fetus

HLC 6 - Dr Mark Sullivan (m.sullivan@imperial.ac.uk)

The placenta

Introduction

- The placenta is the exchange surface between mother and fetus during development.
- It is the primary site of nutrient and gas exchange between mother and fetus
- The placenta is a fetal-materno organ that has two components
  - Fetal part that develops from the chorionic sac (formed by the villous chorion). The chorionic villi that arise from it project into the intervillous space containing maternal blood
  - Maternal part that is derived from the endometrium
- The human embryo's outer cellular surrounding layer is the chorion. Within this two important sets of embryonic membranes develop and play important roles in development.
  - The amnion develops as a cavity within the inner cell mass.
    - It grows and fills with fluid and provides a space into which the embryo projects during folding and within which its further development proceeds.
    - Ultimately folding means that the embryo is only attached to the chorion by a narrow stalk; the part of this stalk that is within the amnion elongates to form the umbilical cord containing the arteries and vein that carry fetal blood between the fetus and the placenta.
  - The other important membrane is an outgrowth from the gut tube endoderm called the allantois.
    - This makes contact with the chorion and, through development of blood vessels at the site of contact, provides the circulatory link between the embryo and the chorionic surface adjoining the endometrium (the uterine lining).
    - The fetus floats weightlessly in the amniotic fluid, protected from injury by this liquid cushion.
- During the early stages of labour the amnion ruptures so that the amniotic fluid escapes through the birth canal (breaking of the waters).
- Premature rupture of the amnion, thought to be associated with abnormal features of the molecular organisation of the amnion’s connective tissue, is an occasional cause of premature labour.

What is the placenta?

- A disc of fetal tissue (weighing ~500g by end of pregnancy)
- Interface between maternal and fetal vascular systems
- Transporter
- Anchor
- Biosynthetic factory (cf. liver) – releases hormones
- Immunological conundrum (genetically distinct from mother)
Development of the placenta

Early placental development is characterized by the rapid proliferation of the trophoblast and development of the chorionic sac and chorionic villi.

EARLIEST CONTACT

- Known as scheme
- A cavity forms in the morula converting into a blastocyst consisting of an embryoblast (inner cell mass), blastocystic cavity and a trophoblast (outer cell mass)
- The trophoblast goes on to form the embryonic party of the placenta
  - It differentiates into two layers and starts to invade the endometrial epithelium and underlying connective tissue
  - This underlying connective tissue contains endometrial glands and capillaries

The epithelial layer of the trophoblast (the cytotrophoblasts) fuse to form the syncytiotrophoblast
  - It burrows through the uterine epithelium to implant the conceptus within the wall of the uterus.
  - It branches into invasive finger-like projections (chorionic villi). These:
    - break down the connective tissue which surrounds them.
    - Erode their way into the endometrium during implantation
    - Branch to form several generations of smaller villi
    - Progressively acquire cores of connective tissue rich in blood vessel connected to the fetal circulation through umbilical vessels
    - Lie in a pool of maternal extracellular fluid until the 10th week of pregnancy
  - However, this can be very dangerous to the mother, and in extreme cases could cause death. If the syncytiotrophoblast breaks off into the mothers blood, it could end up in the lungs (for example), i.e. pulmonary embolism, and cause death. This is hormone dependent, however. In this way, the syncytiotrophoblast is very much like a cancer cell or malignant tumour, in the way it invades the uterine wall.
  - The syncytiotrophoblast also breaks down the blood vessels and in doing so gains access to the mothers blood.
  - It invades and destroys small vessels of uterine lining so that maternal blood flows through the intervillous space.
  - It widens the uterine arteries to increase blood flow.

Decidualisation - This is the normal response of the endometrium to implantation.
  - It modifies uterine arteries to ensure adequate blood flow.
It provides protection mechanisms against rejection of the foetoplacental tissue by the mother’s immune system.

**ABNORMAL IMPLANTATION SITES**

- Usually the conceptus implants in the upper part of the uterus, often in the posterior wall.
- However, serious problems may arise if implantation is at an abnormal site.
  - Implantation in the lower part of the uterus, too close to the cervix, is called **placenta praevia** (= placenta in the way).
  - This causes partial or complete obstruction of the birth canal with the risk of placental detachment during labour, which may cause death of the mother through haemorrhage.
  - At labour, lower portion of uterus stretches so can get early detachment of the placenta and increased risk of bleeding.
- Fortunately this risk is usually identified by ultrasound scanning during pregnancy, allowing a controlled delivery through elective caesarean section.

- Sometimes abnormal implantation is outside the uterus – this is called **ectopic pregnancy** (ectopic means “out of place”).
  - The commonest form is a tubal pregnancy, (in one of the uterine tubes) which if untreated will lead to internal haemorrhage when the tube ruptures.
- Some ectopic pregnancies involve implantation on organs within the peritoneal cavity, another situation fraught with risk.
- Ectopic pregnancy often results in a low output of placental hormones, which may be detected though a weakly positive pregnancy test.
- Termination of the pregnancy is the only safe course of action.
SUMMARY SO FAR

- **Day 9**
  - Cytotrophoblast cell layer proliferates to from multinucleated (giant cell type) layer - syncytiotrophoblast – no cell walls in between nuclei
  - No direct contact with enlarged maternal blood vessels
  - Lacunae within syncytiotrophoblast contain fluid (these are gaps in syncytium; role unknown, but are starting points from intervillus spaces)

- **Day 13**
  - Lacunae enlarge and bump into and mix with maternal capillaries as embryo enlarges
  - 1st blood contact – syncytium
  - Cytotrophoblast starts to form finger like processes which start to block these connections with the maternal blood
  - Secretions from glands come into place

- **Day 21**
  - Villus develops
  - Blood supply on fetal side develops
  - Syncytium no longer outer surface
    - Cytotrophoblast cells grow through the syncytium – anchorage points – and form contact with the blood supply
    - Grow outside and spread sideways to form the outer cytotrophoblast shell
  - Embryo undergoes gastrulation

- **Day 28**
  - Cytotrophoblast on outside continues to proliferate and cut off connections between the maternal blood supply and the lacunae
  - Placental tissue on top, followed by cytotrophoblast shell
  - Red area in middle is occluded arteries by the shell
  - In absence of blood supply, embryo is fed with nutrients by hypertrophy of endometrial glands
  - This is thought to occur in early development as embryo hypersensitive to oxygen tension
  - Not hypoxic – protects embryo from free radical damage
  - Last until ~10-12 weeks then blood supply kicks in

FURTHER DEVELOPMENT

- as the syncytiotrophoblast erodes the uterine blood vessels, they come to lie in a slowly moving pool of maternal blood filling the intervillous spaces.
The maternal blood vessels are eroded right back to the arteries and veins so that the syncytial surface is washed with maternal blood unconfined by vessels.

In this haemochorial type of placenta, there is no maternal component to the barrier between the maternal and fetal blood circulations.

- By the end of third week exchanges between the mother and fetus are established
- Placental circulation similar to lungs – veins carry oxygenated blood etc
- Blood leaving fetal heart receives oxygen from the maternal blood supply at the placenta
- A complex vascular network is established in the placenta by the end of the 4th week, which facilitates the maternal-embryonic exchanges
- villous chorion is produced as villi closer to the conceptus rapidly increases in number, branch profusely and enlarge
- The fetal part of the placenta is the attached to the maternal part by the cytotrophoblastic shell, the external layer of trophoblastic cells on the maternal surface of the placenta

Development of blood supply

- Endometrial arteries and veins pass freely through gaps in the cytotrophoblastic shell and open into the intervillous space
- The intervillous space of the placenta, which contains maternal blood, is derived from the lacunae that developed in the syncitiotrophoblast during the 2nd week of development
- This large blood filled space results from the coalescence and enlargement of the lacunar networks
- Maternal blood enters the intervillous space from the spiral endometrial arteries
- The spiral arteries pass through gaps in the cytotrophoblastic shell and discharge blood into the intervillous space
- This large space is drained by the metrial veins that also penetrate the cytotrophoblastic shell
- The blood carries nutrients and oxygen to the fetus and carries away waste products
- No direct contact between fetal and maternal blood – fetal tissue in between
acenta is top layer – cytotrophoblast shell underlying it
- Endometrium invaded until spiral arteries reached
- Arteries initially occluded by cytotrophoblast cell plugs
- These arteries are remodelled by cytotrophoblast cells which move into the arteries and replace the cells
- The blood vessels therefore lose their ability to respond to vasoconstrictors or dilators
- Once the plugs disintegrate the conceptus becomes exposed to maternal blood supply (at higher pressure) (after 10-12 weeks)

- Remodelled arteries contain trophoblast cells on the inside which results in a wider lumen
- Provides large volumes of maternal blood to the fetus – lost all vasoresponsiveness
- These vessels however do need to shut down at the end of pregnancy
- Upstream often retain vasoresponsiveness – constrict at pregnancy
- Remember these vessels are open ended

Placental circulation
- The branch chorionic villi of the placenta provide a large surface area where materials may be exchanged across the thin placental membrane interposed between the fetal and maternal circulations
- The circulations of the fetus and the mother are separated by the placental membrane consisting of extrafetal tissues

Fetal placental circulation
Poorly oxygenated blood leaves the fetus and passes through the umbilical arteries to the placenta. At the site of attachment of the umbilical cord to the placenta, these arteries divide rapidly into several radially disposed chorionic arteries that branch freely in the chorionic plate before entering the chorionic villi. The blood vessels from an extensive arteriocapillary-venous system within the chorionic villi which brings the fetal blood very close to the maternal blood. This system provides a large surface area for exchange. The oxygenated fetal blood in the fetal capillaries passes into fetal veins that follow the chorionic arteries to the site of attachment of the umbilical cord. They converge here to form the umbilical vein which carries oxygenate blood to the fetus.

The placental membrane
- Consists of extrafetal tissues separating the maternal and fetal blood.
- Until approx 20 wks, the placental membrane consists of 4 layers.
- Synvotiotrophoblast, cytotrophoblast, connective tissue of villus and endothelium of fetal capillaries.
- After 20° wk, histological changes occur in the branch villi that result in the cytotrophoblast in many of the villi becoming attenuated.
- Eventually the cytotrophoblastic cells disappear over large areas of the villi, leaving only thin patches of the synctiotrophoblast.
- This occurs as proliferation rate can’t keep up as fetus develops.
- As a result the placental membrane consists of three layers in most places.
- Therefore the fetal capillaries become closer to the syncytium.
- Transport efficiency increases.

Placental blood flow: problems
- The fetus depends entirely on exchange between the maternal and fetal blood for its supply of oxygen and nutrients and for the elimination of metabolic wastes.
- Insufficiency of either maternal or fetal blood flow through the placenta will impair fetal growth and may threaten fetal survival or constitute a risk factor for stroke, diabetes and other conditions in adult life. So-called placental insufficiency is therefore of great concern.
- During pregnancy the maternal blood flow through the uterus increases greatly; by term it is about one-sixth of the total cardiac output – around 0.8 litre/min.
Placental bed haemorrhage:

- Some minutes after delivery of the baby, the placenta detaches from the uterine wall and is itself delivered.
- This placental separation depends on contraction of the uterine smooth muscle (myometrium) stimulated by the posterior pituitary hormone oxytocin.
- As the circulation of the decidua has been remodelled by trophoblast cells, placental separation has the potential for fatal maternal haemorrhage.
- Normally myometrial contraction not only detaches the placenta but also clamps the uterine vessels in the placental bed. However, premature or incomplete placental separation can pose a great threat to the mother’s life.

Embryonic and fetal nutrition and placental transfer

- Before implantation the conceptus is supported by metabolism of its own substance, and by oxygen and limited supplies of nutrients in the secretions from the uterine glands.
- Once implanted, it can absorb nutrients and oxygen from the extracellular fluid of the endometrium including maternal tissue broken down during syncytiotrophoblast invasion.
- At first the intervillous spaces contain only extracellular fluid; the stage at which maternal blood begins to circulate through the placenta was until recently a fairly contentious issue but is now generally agreed to be around 10 weeks after implantation.
- The placental barrier (the villous syncytiotrophoblast and the endothelium of the fetal capillaries) is only about 2 μm thick + provides for rapid diffusion of small or hydrophobic molecules such as water and oxygen.
- Materno-fetal oxygen transfer is normally limited by blood flow rather than by permeability.
- However, many nutrients such as glucose and amino acids cross the placenta by passive carrier-mediated mechanisms.
- A few large molecules, notably maternal antibodies of the IgG class, cross the placenta by receptor-mediated transcytosis to provide the newborn with an important level of passive immunity.

Exchange

- **Products in:**
  - Glucose, amino acids, lipids, oxygen
  - Peptides and proteins
- **Products (waste) out:**
  - Carbon dioxide
  - Metabolites

Exchange processes

- Diffusion
  - Concentration gradient
  - Additional mechanisms (higher affinities)
- Facilitated diffusion
- Active transport
- Receptor-mediated
- Aqueous or lipid-specific
Endocrine function of the placenta

The successful achievement of implantation needs to be signalled to the maternal endocrine system so that the menstrual cycle stops and pregnancy is supported. Central to this is the secretion by the trophoblast of the glycoprotein human chorionic gonadotropin (hCG). Subsequently the placenta secretes a diverse range of known and suspected hormones including both proteins and steroids.

Maternal tolerance of the placenta - Immunoregulation

- The human placenta is of fetal origin and is therefore “foreign” and at risk of rejection by the maternal immune system.
- The villi are in contact with maternal blood after week 10 and are therefore in contact with maternal immunologically competent cells, while the placentomal base is in contact with the tissues of the uterus.
- The presence of the placenta induces cellular responses called decidualisation in the underlying endometrium, which serve to damp down the local maternal response to the foreign antigen of the placenta.
- In addition, the trophoblast surface carries an unusual set of the HLA antigens determining histocompatibility – in particular it expresses HLA-G, which suppresses local maternal rejection responses.

Birth

HLC 7 - Dr Mark Sullivan (m.sullivan@imperial.ac.uk)

Intro to birth

What is birth? Process involving the delivery of the baby from the uterus (also known as parturition), and the delivery of the placenta (this is more commonly known as afterbirth)

Changes required for birth include:

- Changes in cervix – softens (ripening + dilatation), and stretches sideways (thinner + wears away)
- Changes in myometrium – changes from inert to contracting; contractions are coordinated from top downwards (this is known as fundal)
- Changes in foetal membranes – rupture

Characteristics of birth

- Inflammatory process that is present at all gestational age, but stimulated by initiating factors
- Initiating factors may vary with gestational age; including both pre-term and term initiating factors
- Pre-term factors are better known than term initiators
- Clinical correlate: it is easier to initiate birth, but difficult to stop it after its stimulation by the term initiating factors

Labour

- Pregnancy can be considered to have 4 phases:
  - Phase 0 - Not in labour stage (~39 weeks)
  - Phase 1 - Latent stage (~8 weeks)
- Phase 2 - Labour stage (12-48 hours)
- Phase 3 – afterbirth (30 mins)
- Labour then consists of 3 stages (which overlap the phase 2 + 3 of birth)
  - stage 1 (lasts many hours) – contractions and cervical changes take place
  - stage 2 (lasts few hours) – delivery of baby
  - stage 3 (30 min) – placenta is delivered

**Definition:** A process which results in the delivery of baby, following increased myometrial contractions, softening of cervical tissue and stretching of cervix

- Time of labour may vary -
  - <23 weeks of gestation: miscarriage, results in a non-viable infant being delivered
  - 23-32 weeks of gestation: extreme pre-term labour
  - 23-37 weeks of gestation: pre-term
  - 37-14 weeks of gestation: term labour

**Biochemistry of Labour**

- Labour can be thought of as having inflammatory mechanisms, with nuclear factor NFκB acting as the key transcriptional factor in the process.
  - The key components of the inflammatory process (regulated by NFκB) are IL-1beta, IL-8, COX-2, oxytocin +MMP9
- In 50% of foetal membranes, prostaglandin output and COX-2 expression maximally increases **BEFORE** labour
- In all foetal membranes, prostaglandin output and COX-2 expression increases during labour
- This is important, as COX-2 expression stimulates production of PGF2a, which acts in conjunction with oxytocin-R to stimulate myometrial contractions
- PGE2 is critical as it acts in conjunction with MMPs stimulating cervical ripening
- The output of interleukins (cytokines) is also increased from foetal membranes in association with labour
  - IL-1 and IL-6 stimulate COX-2 expression → prostaglandin production and output (which act on both cervix and myometrium)
IL-8 recruits neutrophils to produce MMPs (especially MMP8 + MMP9, which act to induce cervical ripening)

- Platelet activating factor levels also increase near term; this is a component of lung surfactant produced by the foetal signal that lung maturation is occurring
  - This stimulates surfactant protein complex formations

**NB:** Myometrial prostaglandins are also present; although it is unlikely they have a major role

**Related to the stages of parturition**

- **Phase 0** – not in labour – progesterone and HCG (human chorionic gonadotrophin) maintain myometrial repression
- **Phase 1** – latent stage – cortisol, corticotrophin-releasing hormone (as well as CRH binding factor protein) and oestrogens stimulate myometrial activation
  - There is also progesterone withdrawal, as well as an increase in PAF production from foetal lungs
- **Phase 2** – labour – see above – inflammatory process which causes biochemical activation of the myometrium, leading to myometrial contractions and cervical ripening
  - Oxytocin release from the neurohyophysial axis also causes contractions

**NB:** during labour, there is no change in myometrial oxytocin, but during placental delivery the number of oxytocin receptors increase. However other myometrial contraction associated proteins (e.g. connexins) are increased during labour

**Role of progesterone and the progesterone receptor (PR)**

- During labour, progesterone (which has maintained the myometrium during pregnancy) undergoes permissive changes so that labour can occur. These include:
  - Functional withdrawal
  - Increased oestrogens
  - Altered progesterone cycle patterns
  - Overall less effect
- There is a mutually negative interaction between NFκB and the progesterone receptor whereby PR may inhibit the inflammatory process of labour (especially PR-beta). During labour, there is a co-ordinated up-regulation of NFκB which explains many of the changes in the tissue biology in labour, but also results in changes to PR:
  - The ratio of PR-beta to PR-alpha receptors also decreases
  - There is a change to PR which also leads to functional progesterone withdrawal, removing its inhibitory effects on inflammation
HLC - Embryonic Task Sheet

Question 1

1. Precisely name the two gametes that fuse together at the start of human life.

Oocyte & spermatocyte

a. When fusion has occurred and the female gamete completed its second meiotic division, what do we call the resultant structure?

After fertilization, the oocyte completes its 2nd meiotic division and two pronuclei form.

The male and female pronuclei will intermingle and form a diploid cell, which undergoes mitotic divisions forming a morula.

Question 2

2. Give a brief description of where implantation of the conceptus occurs.

Implantation usually occurs in the anterior or posterior portions of the uterus.

a. If implantation does not occur in the correct place, give an example of where the developing embryo might implant.

In the fallopian tube wall

b. If implantation occurs in the wrong place what type of pregnancy ensues? Can you suggest complications that might occur as a result of this?

As embryonic development continues, and the embryo increases in size, there is an increased risk of rupturing the fallopian tube.

This can lead to severe internal bleeding, which in turn can lead to hypovolaemic shock.

Question 3-5

3. What mechanisms are used to achieve implantation of the developing embryo into the maternal endometrium?

The blastocyst first needs to get rid of its zona pellucida, and then the blastocyst adheres to the endometrium.

The trophoblast cells protrude into the endometrium, which signals the endometrium to its presence (e.g. changing the nature of the cytoskeleton to allow invasion). These cells then differentiate to form syncytiotrophoblast cells.

The syncytiotrophoblast cells secrete enzymes that allow the blastocyst to embed into the uterine wall. The syncytiotrophoblast is so named because there is a lack of cell boundaries, whereas the cytotrophoblast cells then surround the inner cell mass.

Eventually the syncytiotrophoblasts come into contact with maternal blood and form chorionic villi.

4. What is the uterine endometrium?

The inner membrane of the mammalian uterus
5. Why can implantation only occur at certain stages of the menstrual cycle?
During the menstrual cycle, the endometrium forms a thick, blood-vessel rich, glandular tissue layer which represents the optimal environment for the implantation of a blastocyst. At other stages of the menstrual cycle, the endometrium exists only as a thin membrane with no present epithelium or glands therefore is too hostile of an environment for blastocyst implantation.

**Question 6**

6. What is the zona pellucida?
A glycoprotein membrane surrounding the plasma membrane of an oocyte

   a. **State what its functions are.**

      - Supports communication between oocytes and follicle cells during oogenesis
      - Protects oocytes
      - Essential for oocyte death and fertilization
      - Regulates interaction between ovulated oocytes and spermatozoa – the zona becomes specialised once a sperm has traversed it, blocking all others from entering
      - 5 days after fertilization, the zona pellucida degerates and decomposes to be replaced by the underlying layer of trophoblastic cells.

**Question 7**

7. What two cell types constitute the blastocyst?
Embryoblast – inner cell mass
Trophoblast – surrounds embryoblast and blastocyst cavity

   a. **What is the fate of each cell type?**

The embryoblast will form the embryo proper

The trophoblast will form surrounding supportive tissues, and will differentiate to form the syncyiotrophoblast and cytotrophoblast during implantation.

**Question 8**

8. Describe how the inner cell mass gives rise to epiblast and hypoblast
Just prior to gastrulation, the inner cell mass delaminates: The cells of embryoblast differentiate into two layers: the hypoblast layer, consisting of small cuboidal cells, and the epiblast layer, consisting of high columnar cells. The two-layered plate that will differentiate into the embryo is called the embryonic disc. The blastocyst cavity then becomes the exocoelomic cavity (primitive yolk sac), and the cavity above the epiblast becomes the amniotic cavity.

   a. **Describe how the third germ layer of the embryo is formed**

The definitive mesoderm is formed by the invaginating epiblast which has travelled cephalically (towards the head) and laterally from the primitive streak. The remaining epiblast cells form the ectoderm.

   b. **Describe how the extra-embryonic mesoderm of the embryo is formed and be aware that this is a controversial area.**
The hypoblast is then lost, and the endoderm is derived from the mesoderm.

However the extent to which the endoderm is derived from the mesoderm is debated.

**Question 9**

9. Briefly describe the tissues that the 3 layers of the embryonic trilaminar disk are destined to form.

- Ectoderm → skin, nervous system
- Mesoderm → skeleton, muscle, kidney, heart, blood
- Endoderm → gut, liver, lungs