Fertilization and embryogenesis

1. Ovulation and fertilization
2. Assisted reproduction
3. Early development of the human embryo
   - Cleavage and blastogenesis
   - Implantation
   - Bilaminar germ disc
   - Trilaminar germ disc (Gastrulation)
Menstrual cycle

- menstrual cycle (28 days)
  - phases:
    - menstrual – 3-5 days (menstruation)
    - proliferative (follicular) phase – 5-14 day
    - secretory (luteal) phase – till 26-27 day
    - premenstrual (ischemic) phase – 1-2 days

fertile window – ~7 days

the time from 5 days before until 1–2 days after ovulation
Menstrual vs. ovarian cycle

Ovarian Cycle (Follicle Development)
- Follicular Phase
- Luteal Phase

Menstrual Cycle (Uterine Lining Development)
- Menstruation
- Proliferative Phase
- Secretory Phase

Ovarian cycle:
- Primary follicle
- Secondary follicle
- Vesicular follicle
- Ovulation
- Corpus luteum forms
- Regression

Gonadotropin levels:
- FSH
- LH

Ovarian hormone levels:
- Estrogen
- Progesterone

Uterine cycle:
- Menstrual flow
- Functional layer
- Basal layer

Days:
- Follicular phase
- Ovulation
- Luteal phase

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Menstrual vs. ovarian cycle

**Ovarian Cycle**
- Low estrogen at the end of menses

**Follicular Phase**
- Follicle growth
- Increase in estrogen

**Ovulation**
- LH surge
- Release of mature follicle
- Progesterone surge

**Luteal Phase**
- Corpus luteum formation
- Progesterone production

**Uterine Cycle**
- Proliferative phase: influenced by estrogen, the thickness of the endometrium rapidly increases
- Secretory phase: influenced by progesterone, the lining becomes highly vascular and edematous

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Ovulation

- **Ovulation:**
  - The process by which an oocyte is released from the Graafian follicle
  - A cyclic process, blocked during pregnancy
  - Takes place around 14-15 day
  - Preovulatory Graafian follicle
  - Liberation of the ovum
  - Stigma
  - Corona radiata
**Fertilization**

- **fertilisatio (fecundatio):**
  - the process by which the male and female gametes fuse
  - occurs in the ampulla of the uterine tube
  - preceded by insemination – the introduction of sperm into the female reproductive tract

- **main results of fertilization:**
  - restoration of the diploid number of chromosomes
  - determination of the sex of the new individual
  - initiation of cleavage
  - without fertilization the oocyte degenerates 24h after ovulation!
Fertilization requires ...

- penetration of cervical mucus
- capacitation
- penetration of corona radiata (for which capacitation is necessary)
- dissolution of zona pellucida
  - Sperm receptor
  - Acrosomal reaction
- penetrating the egg’s plasma membrane
  - sperm-egg adhesion
Insemination and capacitation

- **insemination:**
  - an internal process in the female genitalia
  - the introduction of sperm into the female reproductive tract
  - ejaculation

- **capacitation:**
  - duration ~ 7h
    - removal of a glycoprotein coat and seminal plasma proteins
  - acrosome reaction - release of:
    - hyaluronidase ⇒ penetration of the corona radiata barrier
    - trypsin-like substance ⇒ digestion of the zona pellucida
    - acrosin (zonalysin) ⇒ helps the spermatozoon cross the zona pellucida
Preconditions of fertilization:

- Capacitation of sperms
  - a period of conditioning in the female reproductive tract

- Acrosome reaction - release of:
  - Hyaluronidase → penetration of the corona radiata barrier
  - Trypsin-like substance → digestion of the zona pellucida
  - Acrosin (zonalysin) → helps the spermatozoon cross the zona pellucida

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Fertilization

phases:
- penetration of the corona radiata
  - 300-500 spermatozoa reach the site
  - only one penetrates
- penetration of the zona pellucida
  - inner acrosomal membrane dissolves
  - zona reaction – avoids consequent penetration
- fusion of oocyte - sperm cell membranes
  - cortical reaction – polyspermy is prevented
  - resumption of second meiotic division ⇒ female pronucleus
  - stage of male and female pronuclei
  - metabolic activation of the egg
Assisted reproduction

- **in vitro fertilization (IVF):**
  - a process by which egg cells are fertilized by sperm outside the womb, *in vitro*
  - provides the opportunity to alleviate infertility from a variety of causes
  - to karyotype fertilize ova as a means of averting chromosomal anomalies
  - disadvantage of the technique – the low success rate (20%) of the procedure
  - four or five ova are collected, fertilized, and placed in the uterus ⇒ multiple births

- **gamete intrafallopian transfer (GIFT):**
  - introduction of oocytes and sperm into the ampulla of the fallopian tube

- **zygote intrafallopian transfer (ZIFT):**
  - fertilized oocytes are placed in the ampullary region of the fallopian tube

- **intracytoplasmic sperm injection (ICSI):**
  - in severe male infertility:
    - a single sperm is injected into the cytoplasm of the egg to cause fertilization
    - an alternative to using donor sperm for IVF
Cleavage

- **segmentatio** - a series of mitotic divisions:
  - begin of the process ~30 h
  - duration – 3-4 days

- cleavage division:
  - total cleavage
  - unequal cleavage
    - microblastomeres
    - macroblastomeres
  - asynchronous

- main events:
  - increase in number of cells in the embryo
  - decrease in size of cells (blastomeres)
  - compaction
  - formation of a morula (mulberry)
    - 16 blastomeres
    - ~3 days after fertilization
Blastogenesis

- **Blastocyst formation:**
  - inner cell mass - embryoblast
    - embryonic pole
    - abembryonic pole
  - outer cell mass - trophoblast
    - cytotrophoblast
    - syncytiotrophoblast

- **disappearance of the zona pellucida**

- entering the uterine cavity
  - 4-5 day
Implantation

- **Implantacio** (Lat. *in*, within + *plantare*, to plant)
- **Nidation, nidacio** (Lat. *nidus*, nest)

- **Begin of the process – 6-7 day**
- **Duration – ~40 h**

**Mechanism:**
- **Adhesion:**
  - Cytotrophoblast
  - Syncytiotrophoblast
- **Invasion:**
  - Erosion of the uterine mucosa (endometrium)
  - Embryoblast – bilaminar germ disk
    - Amniotic cavity
  - Trophoblast – primary villi
    - Chorionic cavity
Implantation

- implantation sites:
  - normal sites of implantation ("plug"):
    - uterine cavity
      - posterior uterine wall
      - lateral uterine wall
      - superior cervix
    - abnormal implantation sites:
      - uterine (Fallopian) tube (tubal pregnancies) - 98%
        - infundibular tubal
        - ampullar tubal
        - isthmic tubal } extraterine (ectopic) pregnancy
      - cervical canal (cervical pregnancies) - <1% (placenta previa)
      - ovaries (primary ovarian pregnancy)
      - mesentery
      - abdominal cavity (abdominal pregnancies)
Bilaminar germ disk

- 2nd week of development:
  - epiblast layer
  - hypoblast layer

- epiblast ⇒ amniotic cavity:
  - outer amnioblasts ⇒ amnion
  - inner amnioblasts ⇒ embryo

- hypoblast ⇒ yolk sac:
  - primitive yolk sac (exocoelomic cavity)
  - secondary yolk sac (definitive yolk sac)
Bilaminar germ disk

- **extraembryonic coelom** ⇒ chorionic cavity
  - somatopleuric mesoderm
  - splanchnopleuric mesoderm

- **connecting stalk** ⇒ umbilical cord

- **trophoblast – lacunar stage**
  - primary villi
  - maternal sinusoids
  - primitive uteroplacental circulation
Trilaminar germ disk

- **3rd week of development:**
  - **gasrtulation:** formation of all three germ layers
  - formation of primitive streak
    - primitive (Hensen's) node
    - primitive pit
  - proliferation and invagination of the epiblast ⇒ formation of **three germ layers**:
    - definitive **ectoderm**
    - intraembryonic **mesoderm**
    - **endoderm**

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[Diagram of germ layers and development stages]

- Primitive streak
- Epiblast
- Mesoderm
- Endoderm
- Notochord
- Extraembryonic mesoderm
- Intraembryonic mesoderm
- Buccopharyngeal membrane
- Yolk sac
- Amnioblasts
- Invaginating mesoderm cells
- Hypoblast
- Epiblast
- Detaching cells
- Primitive node
- Epiblast
- Hypoblast
- Notochord
- Endoderm
- Intraembryonic mesoderm

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[Image of a cell with various labeled parts]
Trilaminar germ disk

- formation of notochord:
  - prenotochordal cells ⇒ prechordal plate
  - bilaminar notochordal plate
  - definitive notochord ⇒ the basis of the axial skeleton (vertebral column)
Establishment of the body axes

- **anteroposterior axis:**
  - cells at the anterior (cranial) margin of the embryonic disc (anterior visceral endoderm – AVE)
    - head-forming genes, including **OTX2**, **LIM1**, and **HESX1** and the secreted factor **cerberus**
    - **chordin**, **noggin**, and **follistatin** (activin-binding protein) and **bone morphogenetic protein 4 (BMP4)**
  - in more caudal regions
    - **brachyury (T)** gene

- **left-right axis:**
  - **FGF-8**, secreted by cells in the node and streak
  - induces **Nodal** and **Lefty-2** expression on the left side
  - these genes upregulate **PITX2**, a transcription factor responsible for left sidedness
Growth of the embryonic disc

- **embryonic disc:**
  - flat and almost round initially
  - elongated - a broad cephalic and a narrow caudal end

- expansion of the embryonic disc:
  - mainly in the cephalic region - by a continuous migration of cells from the primitive streak region in a cephalic direction
  - the region of the primitive streak remains more or less the same size
  - invagination of surface cells in the primitive streak and their subsequent migration forward and laterally continues until the end of the fourth week
  - the primitive streak shows regressive changes, rapidly shrinks, and soon disappears
  - in the cephalic part, germ layers begin their specific differentiation by the middle of the third week
  - in the caudal part, differentiation begins by the end of the fourth week
Thank you...