

## 1. Introduction. Gametogenesis. Fertilization.

### Embryology (greek *ἔμβρυον*, embryo): prenatal ontogeny

- progenesis: fertilization, cleavage, implantation, gastrulation (formation of three germ layers), till the week 4
- embryogenesis: organogenesis, till the week 8
- fetal period: from week 9 until birth

### Processes involved in embryogenesis

- cell division
- cell growth
- cell differentiation
- morphogenesis (anatomical structures change in shape): migration of cells, invagination of cell layers, folding

### Common morphogenesis mechanisms

- mesenchymal:
  - condensation of cells
  - proliferation and cell death
  - migration
  - synthesis of extracellular matrix
  - growth
- epithelial:
  - delamination (splitting layers)
  - cells changing their shape and size
  - migration
  - intercalation
  - proliferation and cell death
  - matrix synthesis and degradation

### Cell to cell interactions in embryology

- induction: started by inducers, transmitted via signal molecules to target cells equipped with receptors (having competence to respond)
- transport of signal molecules: endocrine, paracrine and autocrine

### Genotype and phenotype

- genotype = inherited instructions encoded in DNA of an organism
- phenotype = organism's observable characteristics
- forming the phenotype
  - increasing complexity
  - ruled by the genotype and epigenetics

### Epigenetics

- changes in gene expression (gene in/activation), DNA methylation, chemical modification of nuclear histones, non-coding RNA with regulation function, pre-mRNA splicing, posttranslational modification of proteins
- cell-to-cell contacts, determination of cellular position, chemical gradients
- radiation (fields), movements, perfusion, intrauterine conditions etc.
- genomic imprinting: an epigenetic phenomenon independent from Mendelian inheritance;
  - certain genes are expressed in a parent-of-origin-specific manner
  - the allele from the father is silenced and only the allele from the mother is expressed
  - or the allele from the mother is silenced then only the allele from the father is expressed
  - important for normal embryonic development; disorders: Prader-Willi syndrome (hypotonia, obesity, hypogonadism) and Angelman syndrome (epilepsy, tremor, constantly smiling facial expression, "puppet children")

### Embryology in the medical curriculum

- understanding human anatomy, human anatomical variability, differentiation and growth of cell populations, tissues and organs
- links between organogenesis, developmental disorders and birth defects are necessary for pathology, imaging methods, paediatrics, obstetrics, surgery and internal medicine
- clinical embryology and assisted reproductive techniques/technology (ART): diagnosis and treatment of infertility; assessment of spermogram, hormone therapy; IVF-ET (in vitro fertilization followed by embryo transfer); GIFT – gamete intrafallopian transfer; ICSI – intracytoplasmic sperm injection

### Embryology and ethics

- preimplantation diagnostics, selection of gametes and embryos
- gamete donation (anonymous vs. non-anonymous), "womb for rent"
- more genetical and biological parents: sperm donors, oocyte donors, but also donors of oocyte nuclei implanted into previously enucleated oocytes retaining their mitochondrial DNA
- what is to be done with surplus embryos?
- embryonic stem cells used in tissue therapy
- multiple pregnancy following assisted reproduction
- coping with biological incompatibility of infertile pairs – consequences for further generations
- greater risk of embryonic and fetal developmental defects in advanced age mothers > 35 years (chromosomal aberrations etc.)
- screening of developmental defects, pregnancy termination

### Comparative embryology

- human developmental anatomy linked to common ancestors with other primates, placental mammals, vertebrates, chordates, deuterostomes and triblastics
- importance for experimental embryology and testing teratogens

- translation of knowledge from animal models into human medicine
- heterochrony: changes in change in developmental timing during evolution, e.g., further postnatal growth of CNS continues for a longer time and is more pronounced in human than in other primates
- neoteny (juvenilization) as a slowing of the rate of development with the consequent retention in adulthood of a feature or features that appeared in an earlier phase in the life cycle of ancestral individuals; pedomorphosis as retention by adults of traits previously seen only in the young; when compared to other primates, humans have neotenic and pedomorphic features, such as flat and broad face, reduced body height, small nose, small teeth and jaws, relatively short limbs, large eyes

### **History of embryology**

- W. Harvey (16<sup>th</sup>/17<sup>th</sup> century) – “Omne vivum ex ovo”
- A. van Leeuwenhoek (17<sup>th</sup>/18<sup>th</sup> century) – sperm cells drawn
- C. F. Wolff (18<sup>th</sup> century) – epigenetics affects differentiation
- K. E. von Baer (19<sup>th</sup> century) – human oocyte
- E. Roux, E. Driesch, H. Spemann (19<sup>th</sup> century) – experimental embryology, embryonic differentiation of organs, hypothesis on embryonic organizers
- J. G. Mendel (19<sup>th</sup> century) – phenotype is based on inheritance (genes); genes occur in two alternative forms = alleles; genes are subjected to recombination within the germ cells (gametes); the phenotype is based on a combination of genes; attention to the laws of Mendelian inheritance was paid after 1900 by H. de Vries, C. Correns, E. Tschermak
- O. Hertwig (1875) – only one sperm cell takes part in fertilization; 1890 – phases of meiosis
- T. Avery (1944) – DNA identified as the molecule carrying the genes
- J.D. Watson, F. H. Crick (1953) – DNA structure revealed
- L. Wolpert (20<sup>th</sup> century) – positional information and pattern formation is regulated by molecules working as organizers in embryonic development

### **Nobel prizes and embryology**

- 2012: reprogramming of mature cells back into pluripotent cells resembling early embryonic period (Yamanaka’s transcription factors, intended to be used in cell therapy techniques)
- 2010: development of in vitro fertilization
- 2009: how chromosomes are protected by telomeres and the enzyme telomerase
- 2007: introducing gene modifications by the use of embryonic stem cells
- 2006: RNA interference gene silencing by double-stranded RNA
- 2002: genetic regulation of organ development and programmed cell death
- 2001: key regulators of the cell cycle
- 1998: role of nitric oxide as a signal molecule involved, e.g., in gametogenesis, ovulation and fertilization
- 1995: genetic control of early embryonic development (body axis and segmentation)
- 1986: discoveries of growth and differentiation factors
- 1978: discovery of restriction endonucleases and their application to molecular genetics
- 1960: discovery of acquired immunological tolerance

- 1935: micromanipulation experiments - the organizer effects in embryonic development
- 1933: role of chromosomes in heredity

### Gametogenesis

- spermatogenesis:
  - series of mitotic divisions: primordial gonocytes → spermatogonia → type A spermatogonia (self-renewal) and type B spermatogonia (further differentiation) → primary spermatocytes (two sets of chromosomes, two chromatids per chromosome = 4N DNA content)
  - primary spermatocytes undergo the first meiotic division → secondary spermatocytes (haploid set of chromosomes, two chromatids per chromosome, 2N DNA content) → second meiotic division → spermatids (haploid set of chromosomes, single chromatid per chromosome, 1N DNA content)
  - spermatids mature into spermatozoa via a series of changes named spermiogenesis; this consists from chromatin condensed to heterochromatin; formation of acrosome from the Golgi complex; formation of neck, middle piece and tail; arrangement of mitochondria within the middle piece; shedding most of the cytoplasm);
  - the whole process takes approx. 64 days, supported by Sertoli and Leydig cells, FSH and LH gonadotropic hormones
  - 95% of nuclear histones are temporarily replaced by protamines → more compact chromatin within the sperm head
- oogenesis:
  - primordial gonocytes → oogonia undergo a number of mitotic divisions during the prenatal period, they got surrounded by a layer of flat epithelial follicular cells → primordial follicles are formed, each containing primary oocyte (diploid set of chromosomes, 4N DNA content, the first meiotic division is arrested from the fetal period in the diplotene phase of the prophase)
  - most follicles including the oocytes degenerate (=atresia)
  - maturation of oocytes continues since puberty: in every ovarian cycle, 15-20 follicles grown mature (follicle-stimulating hormone, FSH) → into primary follicles (unilaminar and multilaminar follicles with membrana granulosa and zona pellucida layers) → secondary follicles (with a follicular antrum, cumulus oophorus, theca folliculi interna and externa) → one of the follicles reaches the stage of a tertiary (Graafian) follicle (20-25 mm in diameter)
  - shortly before ovulation, the membrana granulosa cells expand, thus forming an expanded cumulus; thus the cell-to-cell gap junctions between cell projections of cumular cells penetrating the zona pellucida and the oocyte membrane stop inhibiting the oocyte meiosis (the meiotic block) → the primary oocyte finishes the first meiotic division prior to ovulation (managed by the LH) → secondary oocyte (haploid set of chromosomes, 2N DNA content) and a polar body; the secondary oocyte is arrested in metaphase of the second meiotic division until fertilization occurs

### Ovulation

- triggered by luteinizing hormone (LH) (and also by FSH) → the secondary oocyte is released (together with the zona pellucida and corona radiata) into the abdominal cavity → transported to the distal ampulla of the uterine tube; if no fertilization occurs, the oocyte disintegrates within 24 hours
- follicular cells grow and mature into a corpus luteum which secretes amounts of sex hormones (mainly progesterone); without the human chorionic gonadotropin (hCG), the corpus luteum degenerates as a fibrotic scar (corpus albicans);
- if fertilization occurs and the syncytiotrophoblast cells produce hCG, the c. luteum continues to grow and forms the corpus luteum graviditatis (its hormones are necessary for maintenance of pregnancy until 4<sup>th</sup> month)

### Semen analysis: current (and former) criteria according to the WHO

- volume of the semen  $\geq 1,5$  ml ( $\geq 1,5$ -2,0 ml)
- pH 7,2 – 8,0
- sperm count = sperm concentration  $\geq 15$  mil/ml ( $\geq 20$  mil/ml)
- total sperm count  $\geq 39$  mil ( $\geq 40$  mil)
- motility  $\geq 50$  % ; vitality  $\geq 58$  % ( $\geq 75$  %)
- morphology normal form in  $> 4$  % sperm cells ( $> 30$  %)
- leukocytes  $< 1$  mil/ml; autoagglutination  $< 2$  (grades 0–3)

### Fertilization

- 200-300 millions of sperm cells in ejaculate
- approx. 1% will reach the uterus,  $10^1$ - $10^2$  sperm cells reach the oocyte
- fusion of the oocyte and sperm cell (ampullary region of uterine tube)
- capacitation (up to 7 hours) – glycoproteins and seminal plasma removed → acrosome becomes activated
- acrosome reaction
  - hyaluronidase → penetration of the expanded cumulus cells and the corona radiata
  - acrosin → penetration of the zona pellucida
- cortical and zona reaction
  - the head of a sperm penetrates the oocyte membrane
  - mitochondria also penetrate into the oocyte, but are destroyed soon in the proteasome after being marked with ubiquitin
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  - oocyte membrane and zona become impenetrable → prevent polyspermy
- secondary oocyte will finish the meiosis II → female pronucleus formed (and the second polar body)
- the male and the female pronucleus contributes with one allele from each gene
- male and female pronuclei fuse and replicate their DNA (duplication of chromatids) → diploid zygote