

Cultivation of cells and tissues in vitro, significance in medicine

Culture for cytogenetic examination

- chromosomal examination is possible only with full spiralization of chromosomes and after appropriate processing of bb.
- therefore, direct processing is only possible for cells from tissues with high mitotic activity (bone marrow, tumor, chorionic villi), but for most other cells, cultivation is necessary
- the mitotic index is used to determine whether tissue cell culture is necessary and for how long
- **mitotic index = $\frac{bb \text{ in mitosis}}{\text{all tissue bb}} * 100 (\%)$**
- examination of chromosomes in meiosis is not performed as a standard (the exception is the examination of sperm and eggs for the purposes of assisted reproduction)

Cultivation conditions

- cultivation can be short-term (e.g., peripheral blood cells - 72 hours) or long-term (weeks)
- cultured in vials or tubes with culture medium supplemented with serum with growth factors
- the temperature during cultivation is 37 °C, the pH must be constant, for long-term cultures 5% CO₂ in the thermostat atmosphere is also important
- tissue cells are mechanically or enzymatically loosened before cultivation

Cultured cells

- for postnatal examination, mostly peripheral blood lymphocytes are cultured after the addition of the mitogen phytohemagglutinin
- cells from amniotic fluid (amniocentesis - 16 to 18 weeks of gestation), trophoblast cells (trophoblast biopsy 11 to 12 weeks) or lymphocytes from fetal blood (cordocentesis - after 20 weeks) are used for prenatal examination

Processing culture

- addition of colchicine - destruction of the dividing spindle (colchicine is a poison from ocun, its duration of action is shorter for HRT methods, when cells in prometaphase are used)
- action of hypotonic solution 0.075M KCl → loosening of cell contents and individualization of chromosomes
- fixation with a mixture of acetic acid and methanol (1:3)
- dripping the specimen onto a glass slide + staining (e.g., streaking)

Links

- ws: Kultivace buněk a tkání in vitro, význam v medicíně

References

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