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Pšenička M.: Isolation and cryopreservation of early stages of germ cells of tench (*Tinca tinca*)

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A practical technique for isolation and cryopreservation of tench (*Tinca tinca*) (Cyprinidae, Teleostei) early stages of germ cells (GC), including spermatogonia and spermatocytes, is reported for the first time. The germ-line cells possess the ability to differentiate into functional gametes of both sexes. These early stages of germ cells are small enough to be well-suited to cryopreservation, which, together with their high level of plasticity, makes their preservation a promising tool for maintaining genetic resources. Testicular cells were distinguished and separated by Percoll gradient, with the highest proportion of GC (62.2%) obtained from the 30% layer. The concentration and viability of GC were determined, and specific staining (DDX4) for germ cells was used to distinguish GC from somatic cells. Early stages of germ cells were cryopreserved in an extender composed of phosphate buffered saline (pH 8) with 0.5% BSA, 50mM d-glucose, and containing 1.5M cryoprotectant in the pre-programmed PLANER Kryo10 series III using a cooling protocol from +10°C to -80°C at a rate of 1°C/min. The effect of six cryoprotectants – methanol, dimethyl sulfoxide, dimethyl sulfoxide + propanediol (1 : 1), glycerol, ethylene glycol, and dimethylacetamid was assessed, and the results were evaluated by comparing the percentage of viable frozen/thawed GC by ANOVA, Tukey's HSD test ($P < 0.05$). Almost the same viability rates were obtained with no significant differences among tested cryoprotectants, indicating high stability of GC in cryoprotectants. Nevertheless, glycerol at a concentration of 1.5M was associated with the highest survival rate of thawed tench GC ($57.69 \pm 16.85\%$).

Keywords:

germ cells; isolation; transplantation; Percoll gradient; cryoprotectant; viability

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