

Techniques of Embryo Cryopreservation

① → First developed involved the use of dimethyl sulfoxide as a cryoprotective agent.

→ The embryos were cooled very slowly at a rate of 0.3 to 0.5°C/minute, to a temperature of about -60°C before immersion in liquid nitrogen.

② → Then they had to be thawed quite slowly for maximum recovery.

③ → Slow cooling can be terminated at a temperature of around -35°C.

→ After transfer to liquid nitrogen the rate of thawing must be rapid in order to avoid recrystallisation of residual intracellular H₂O, but the survival rate of embryos is equally high.

Process of Embryo Cryopreservation

- I) Embryo cells are taken in cryogenic vial (< 10 ml medium).
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- II) 10 vials are taken, (< 1 ml in each vial).
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- III) Cells are then put into vials (< in saline condition >)
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- IV) Freeze the cells at initial level without using cryoprotectants (< sometimes DMSO is used >) at $1^{\circ}\text{C}/\text{min}$.
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- V) Freeze the cells at -6°C .
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- VI) Again freeze the cells at -80°C for overnight.
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- VII) Formation of ice crystal at this temp. occurs.
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- VIII) Finally put the container at this temperature in the liquid nitrogen at -196°C after 24 hours for future use.

* Frozen Embryos

→ The frozen embryos can simply be thawed and transplanted to recipient as they become ready to transplant.

→ The long term preservation which is afforded by deep freezing also means that embryos can be kept in before export or import.

→ Frozen embryos are also used for genetic banking for conservation of rare or endangered breeds.

→ It is essential to develop Embryo banks to safeguard possible future needs.

→ Example - sheep embryos can be frozen.

→ no satisfactory technique has yet been developed and described for deep-freezing of pig-embryos.

* Advantages of Embryo Cryopreservation

→ Embryo cryopreservation is useful for leftover embryos after a cycle of ~~and~~ in vitro fertilisation.

As Patients who fail to conceive may become pregnant using such embryos without having to go through a full IVF cycle. Or, if pregnancy occurred, they could return later for another pregnancy.

→ It is not necessary to freeze large amount of embryos, only sufficient amount is ~~req~~ required to provide a sufficient number at appropriate stage of oestrous cycle to receive fresh embryos.