

PRAPA MAHAKIT : APPROPRIATE STAGES AND PRESERVATION PERIOD OF
MOUSE EMBRYO IN CRYOPRESERVATION. THESIS ADVISOR : ASSO. PROF.
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This project aims to study the cryosurvival of 2-, 4- and 8-cell mouse embryos subjected to frozen/thawed procedures. Different stages of the mouse embryos were cryopreserved using PROH as the cryoprotectant and rapid freezing method on a programmable biological freezer. Viability of frozen/thawed embryos were assessed by counting the number of embryos developed to blastocyst in vitro. The effect of cryoprotectants used on the cryopreservation of 8-cell mouse embryos was also studied. This was carried out by freezing 8-cell mouse embryos in PROH, DMSO or Glycerol using the rapid freezing method. The survival of embryos in different cryoprotectants were assessed by means of the rate of blastocyst formation in vitro of frozen/thawed embryos. Finally, the effect of the length of storage on cryopreservation of 8-cell mouse embryos was studied. The embryos were frozen and stored for 1, 28 and 56 days in liquid nitrogen [LN₂] and the survival was assessed by culturing thawed embryos to blastocyst stage as above. The viability of blastocysts developed in vitro from frozen / thawed 8-cell embryos was further investigated by transferring into pseudopregnant recipients.

Results showed that frozen/thawed 2-, 4- and 8-cell mouse embryos were able to develop to blastocysts in HTF+20%FCoS medium. The best survival rate was obtained from 8-cell embryos (80.8%). The percentage of blastocyst formation was significantly high ($p < 0.05$) in 8-cell compared to 2-cell or 4-cell embryos. No significant difference in the percentage of blastocyst formation was observed between 2-cell and 4-cell embryos. As a cryoprotectant, PROH gave significantly higher (84.6%) ($p < 0.05$) rate of survival of 8-cell embryos than DMSO (63.2%) or Glycerol (62.5%). No significant difference in the cryosurvival of embryos was noted between the latter two cryoprotectants. Similar survival rates was observed for 8-cell embryos cryopreserved and stored for 1, 28 and 56 day in LN₂. Frozen/thawed 8-cell embryos developed to blastocyst stage in HTF + 20% FCoS were able to implant (30.9%) when transferred to pseudopregnant recipients. However their implantation rate was lower than those obtained from the transferred of blastocysts developed from unfrozen (fresh) 8-cell embryos in vitro.

In conclusion, the present study showed that mouse embryos at the 8-cell stage was the most suitable stage for cryopreservation. Various types of cryoprotectant differently affect the rate of embryo survival. However the latter was not affected by the length of storage time in LN₂. Besides cryopreservation also affect implantation but not culture conditions.