Development *in vitro* of mouse embryonic nuclei fused to chemically enucleated oocytes

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In the present study the possibility of using chemically enucleated oocytes as recipient cytoplasts in nuclear transplantation experiments was investigated. First, zona-free mouse 2-cell stage embryos were employed as a model for the fusion of cytoplasts and karyoplasts to optimize the electrofusion conditions in electrolytes. High fusion (92.5–100%) and development rates to the blastocyst stage (82.4–94.5%) were obtained when the 2-cell stage embryos were exposed to 1 to 4 direct current pulses of 70 V/mm for 30 to 90 μsec, and an alternating current of 5 to 10 V/mm. Under these optimum fusion conditions high fusion rates of cytoplasts and karyoplasts can be achieved in phosphate-buffered saline containing 10% fetal calf serum. Second, the optimum concentrations of etoposide (ETO) and cycloheximide (CHXM) that can lead to high rates of chemical enucleation of mouse oocytes were determined. Concentrations of ETO and CHXM between 36–50 μg/ml were proved the best for chemical enucleation of mouse metaphase I (M I) oocytes. In addition, single blastomeres from late 2-cell stage embryos were aggregated with zona-free chemically enucleated oocytes of different ages and electrofused and cultured to test their developmental ability. The results showed that cytoplasts from chemically enucleated oocytes have detrimental effect on the development of late 2-cell stage blastomeres. Third, the quality of mouse oocytes enucleated chemically at the M I stage was improved by exposing them to 0.75 M sucrose. The results showed that 21.5% of chemically enucleated oocytes exposured to sucrose supported the development of the reconstituted embryos to blastocysts. In addition an attempt was made to enucleate mouse metaphase II (M II) oocytes functionally by exposing them to ETO. The results suggest that mouse M II oocytes can be functionally enucleated by ETO treatment. Last, as a preliminary study to improve the development of the reconstituted embryos by a protective layer such as an artificial zona pellucida (AZP) made of sodium alginate, mouse pronuclear stage embryos were employed as a model to investigate the effects of AZP on *in vitro* development of manipulated and non-manipulated embryos. The results suggest that AZP can be employed to improve the *in vitro* development of the reconstituted embryos.

In conclusion, ETO-treated mouse oocytes can be used as recipients for nuclear transfer. Exposing the ETO-treated oocytes to sucrose is essential to improve the development of the reconstituted embryos. Furthermore, it is also suggested that the development of the reconstituted embryos can be improved by encapsulation with AZP.

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