BTX Applications

Tech-Trends
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Electrofusion of GV to ooplasm

Transfer of germinal vesicle to ooplasm of young mice could not rescue ageing-associated chromosome misalignment in meiosis of oocytes from aged mice

BACKGROUD: Transferring a germinal vesicle (GV) from an aged woman's oocyte into ooplasm from a younger woman has been proposed as a possible way to overcome the problem of age-related decline in female fertility. Here we assessed this possibility by determining whether ooplasts derived from young mice could rescue ageing-associated chromosome misalignment in meiosis of oocytes from aged mice.

METHODS: Three groups of reconstructed oocytes, young GV–young cytoplast (group YY), aged GV–young cytoplast (group AY), and young GV–aged cytoplast (group YA), were created by micromanipulation and electrofusion.

RESULTS: Nuclear transplantation was successful in 89.8–94.4% of GV–ooplast complexes, and maturation rate of the reconstructed oocytes was 93.5–97.9%. Confocal microscopy analysis showed a significantly higher rate (49.2%) of chromosome misalignment in ageing mice than in young mice (16.9%), and 57.1% of oocytes in group AY exhibited chromosome misalignment, while the abnormality rate in groups YY and YA was 16.3 and 16.7% respectively. Calcium imaging showed that the three groups of reconstructed oocytes exhibited a similar pattern of calcium oscillations upon stimulation with bovine sperm extracts. Fertilization rate and developmental capacity to 2-cell embryos were also similar among the three groups of oocytes.

CONCLUSIONS: Our findings suggest that: (i) the ooplasm from young mice could not rescue ageing-associated chromosome misalignment in meiosis of GV from aged mice; and (ii) behaviour of chromosome alignment over metaphase spindle is predominantly determined by GV material.

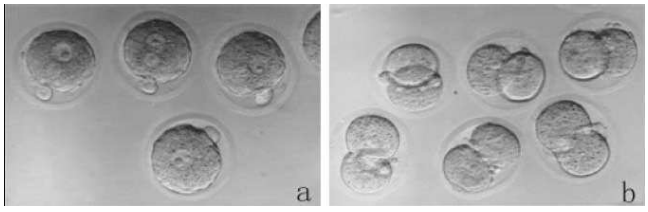

Figure 3. Following IVF, the matured reconstructed ooctyes formed pronuclear stage (a) and developed to 2-cell stgage (b) (£400).

Protocol ECM 2001/ Microslide Chambers

Preparation:
Place groups of 20-30 intact GFP expressing embryos at 1-4 cell stage into a 100mm glass Petri dish filled with chilled HBS (Hepes buffered solution). Using a glass pasture pipette add approximately 20-50µl of acid Tyrode's solution to the embryos and immediately remove them from the solution. Wash the embryos in M2 media extensively to dilute the acid solution and allow embryos to sit in fresh M2 media for 30-60mins prior to experiment

Electrical setting:

Set Alignment Amplitude (AC):	NA
Set AC Time:	NA
Set DC Voltage:	20V
Set DC Pulse Width:	1-2msecSet **Number of Pulses:** 3
Set Number of Series:	2
Chamber:	Flat Electrode 1mm gap (model 484)
Desired Field Strength:	0.2kV/cm

Electroporation Procedure:

Transfectant:	Dilute GFP dsRNA in chilled HBS for a final concentration of 20µg/ml and add 0.5-0.7µl of the dilution to the Flat electrode chamber.
Sample:	Place embryos into the chamber
Temperature:	Buffer for electroporation is chilled prior to experiment
Pulse:	Press Automatic **Start** to Activate Pulse Sequence
Plate:	Following electroporation wash the embryos through 37℃ M2 media and place Into culture media and culture for up to 4 days until they reach morula/blastocyst stage

Results:
95% of the GFP expressing 1 cell embryos and 71% of the GFP expressing 4 cell embryos showed a decrease in the GFP expression

Reference:
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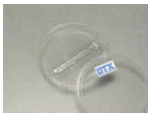

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