Comparison between different combinations of chemical treatment on parthenogenetic activation of mouse oocytes and its subsequent embryonic development

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Combination of different chemicals has been an effective method to activate oocytes. The objectives of this study were to evaluate the optimal: (1) concentration (Experiment 1) and duration (Experiment 2) of strontium chloride (SrCl₂) in the presence of cytochalasin B (CB), (2) secondary activation agent (6-dimethylaminopurine (6-DMAP), cycloheximide (CHX) and CB) in combination with calcium ionophore (Cal) (Experiment 3) and (3) ethanol (EtOH) concentration in combination with 6-DMAP (Experiment 4). There were no significant (P > 0.05) differences in cleavage (86.82–89.06%) and blastocyst (33.42–46.83%) rates when oocytes were treated with various concentrations of SrCl₂ (2–10 mM). Three-hour duration generally showed the highest cleavage (93.83%) and blastocyst rates (60.42%) compared to other durations. Comparing with various combinations, Cal + 6-DMAP showed the highest cleavage at all stages of development and significantly (P < 0.05) higher from other combinations at 8-cell (50.58%), morula (20.29%) and blastocyst (23.28%) stages. When comparing the EtOH concentrations, 8% EtOH gave significantly (P < 0.05) highest cleavage rates at 8-cell stage (39.70%), morula (21.50%) and blastocyst (13.60%) stages followed by 9% and 7% EtOH. In conclusion, 10 mM SrCl₂ + 5 μg/ml CB in calcium-free Chatot Ziemek Bavister medium for 3-h incubation duration gave the highest cleavage rates of parthenogenotes mouse oocytes.

Keywords: embryonic development; mouse oocytes; parthenogenesis; strontium chloride

Introduction

Pre-implantation embryos can be produced in mammals by parthenogenesis, i.e. without involvement of sperm as in normal fertilisation. The main principal step for the success of nuclear transfer for cloning is the efficient method of oocyte activation (Kishikawa et al. 1999). Currently, parthenogenotes have been used in co-transfer experiments for establishment of pregnancies in studies of somatic cell nuclear transfer. Mouse embryos produced by parthenogenetic activation can also be further used in mouse cloning as well as in the production of embryonic stem cells (ESCs). Since the crucial step in successful nuclear transfer protocols is the activation of recipient oocytes (Krivokharchenko et al. 2003), incomplete oocyte activation may result in the inability of pronuclear formation which leads to unsuccessful nuclear transfer. Therefore, it is important to establish an optimal method to activate mouse oocytes in order to proceed to the next step of cloning technique.

Various activation agents have been applied to activate mammalian oocytes during the cloning of mice, sheep, goats, pigs and cattle. However, activation protocols must be optimised for use in each species (Krivokharchenko et al. 2003) as different species have different specific effect on the different kinds of chemicals. Strontium was found to be an effective activation agent for mouse oocytes (Wakayama et al. 1998). It is the only parthenogenetic agent for mouse oocytes that induces repetitive intracellular Ca²⁺ releases in a way similar to normal fertilisation process by sperm (Kline 1996). According to Ma et al. (2005), there were only a few systematic studies carried out to determine the optimal concentration and duration of strontium treatment for mouse oocytes (Ouegai et al. 1999), even though strontium has been used often to activate normal oocytes for analytical studies of oocyte activation (Moe et al. 2004) as well as to activate enucleated oocytes for cloning (Wakayama et al. 1998).

Although many researchers reported the optimal concentration and duration for strontium chloride (SrCl₂), a problem is still faced where the activated mouse oocytes were shrunk after a 6-h incubation in SrCl₂ [10 mM SrCl₂ + 5 μg/ml CB in calcium-free Chatot Ziemek Bavister (CB) medium]. It is not clear whether reduction of SrCl₂ concentration or duration could alleviate the shrinking phenomenon of oocytes, thus increasing the cleavage rates which subsequently determine the optimal concentration and duration of SrCl₂. In addition, it is necessary to determine the effect of combination treatment of intracellular Ca²⁺ stimuli. The objectives of this research were to assess the optimal method for mouse oocyte activation by various combination treatments using SrCl₂, cytochalasin B (CB), cycloheximide (CHX), calcium ionophore (A23187), ethanol (EtOH) and 6-dimethylaminopurine (6-DMAP) as well as to compare the parthenogenotes.