Chimera Production by Embryo Aggregation Method and Cultured *In Vitro* Without Zona Pellucida in Mice

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ABSTRACT. **The objective of this research was to study the developmental competence of single and aggregated embryos develop in vitro and in vivo in relation to the phenotypically aspects of the chimeric mice. Chimeric embryos were produced by aggregation of early stage embryos (8-cell stage) collected from mice with different coat coloration (white and brown). Zona pellucida was removed by exposing the embryo into the medium contained 0.25% pronase. The aggregation of zona-free embryo was done by physically micromanipulation with different composition of embryos collected from white and brown mice. The developmental competence of single zona-free or aggregated embryo in in vitro culture was evaluated morphologically and by counting the total of the cell number of the embryos. The treatment of zona removal and aggregation did not affect the developmental competence of embryo to expanded blastocyst after culture in vitro (P>0.05). The development rate of embryo to expanded blastocyst was 86.49%, 75.00%, 72.22%, 81.82% and 76.92% for normal embryo, single zona-free embryo, aggregated two, aggregated three and aggregated four embryos, respectively. The size of aggregated embryos was bigger (P<0.05) when more number of embryos was aggregated than less number of embryos. Similarly, the total cell number of the aggregated embryos developed to blastocyst was significantly different among the treatment (73.85, 120.50, 160.82 and 220.00 cells per embryo for the single zona-free, aggregated two, aggregated three and aggregated four embryos, respectively). The viability of aggregated embryos develop in vivo was examined by transfer of the aggregated embryos to the pseudopregnant female. Twenty four aggregated embryos were transferred and two chimeric mice were born. These results showed that chimeric mice could be produced by aggregation of early stage embryo and cultured in vitro without zona pellucida.**

Key words: chimera, embryo aggregation, in vivo, in vitro

Introduction

Chimera production is one of the various methods have been used on the developmental biology studies. Chimeric animals are those have two or more cell population containing different genetic materials. Chimeras have proved to be valuable in experiments designed to study the cells lineage pathways in developing embryos, sex determination (McLaren, 1975), cells interaction and rescued lethal phenotype (Surani et al, 1977; Stevens, 1978; Boediono et al, 1999).

In addition, chimeras could be a tool to study genetic modification. Essentially three areas of technology exist for genetic modification namely: pronuclear injection, single genotype animal production and chimeric production (Campbell and Wilmut, 1997). Genetic modification is a recent progress that can be applicable for animal breeding.

Chimera could be produced by introducing cell into the early stage of developing embryo (Hogan et al, 1986; Polzin et al, 1987) or by aggregation of the early stage of developing embryos (Piedrahita et al, 1992; Boediono et al, 1993; Boediono et al, 1999). The termination-known part of late stage embryos or fetus derived from different species could be exchanged and form chimeric animals (Gilbert, 1988).

Several experiments indicated that chimeric embryos possessed some advantages over single embryos. Using interactive behaviour between blastomeres, aggregation enhance the viability of parthenogenetic embryos developed both in vitro and in utero resulted the parthenogenetic derived cells contributed in live chimeric animal (Stevens, 1978; Fundele et al, 1991; Boediono et al, 1999). Although the contribution of parthenogenetic cells origin was less than 20% of the total cell population (Surani et al, 1977). Chimera produced by aggregation method possibly reaches the sex ratio (phenotype) and prefers to be male. It has been reported in mice, from the XX-XY chimeras they were 15% developed to female, 6% intersex and