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In vitro development of bovine cloned embryos using an improved portable CO_2 incubator

T. Suzuki, M. Fahrudin and T. Otoi

The United Graduate School of Veterinary Science, Yamaguchi University, Yamaguchi 753-8515, Japan

Originally, commercial interest in cloning technology on the production of a large number of genetically superior animals for agricultural purposes. However, all of research groups could not overcome embryos cloning inefficiencies and the difficulties in producing multiple generation calves via recloning and, in general, the poor survivability of some cloned calves was and will continue to be a major concern. Along with the lower pregnancy rates of cloned embryos, clearly alone will not become commercially successful on a large scale until these problems are solved.

The objective of this study was to examine the effect of our developed carbon dioxide incubator for producing of cloning embryos using frozen-thawed somatic cell. In this experiment, a portable incubator made of metal (length x width x height=29 x 26 x 21 cm, volume of 15.81) was electrically heated to maintain a chamber temperature of 38.5℃. Incubations were performed in small plastic boxes (15 x 10 x 4 cm=volume 0.61 units) placed in the incubator. The small plastic box was given a negative air pressure (-300mmHg) by aspirating air from it. CO_2 requirement was achieved by adding 5ml of distilled water to the effervescent granules in a small plastic screw cap to release the CO_2 gas.

The donor nucleus were isolated from 7-10 passages of the culture cell lines, and were induced to enter a period of quiescent by serum deprivation for 4-7d before being used as donor karyoplasts. Recipient cytoplasts were prepared from mature oocytes, which were enucleated at 18-20h after the onset of IVM. Nuclear transplantation and fusion were accomplished at 22-24h after the onset of IVM. The fusion between donor nucleus and recipient cytoplasm was initiated by single DC pulse of 1KV/cm for 25μsec. Subsequently, the fused couple was activated parthenogenetically by culturing in CR 1aa medium contained 10μg/ml of calcium ionophore for 5min, and followed by culturing in medium containing 10μg/ml of cycloheximide for 5h. The reconstructed embryos were cultured in CR 1aa medium supplemented with 5% FCS for 8d using our developed CO_2 incubator.

The in vitro development of reconstructed embryos were 64% (125/195), 91% (114/125) for fusion and cleavage rates, respectively, and 47% (57/125) of the reconstructed embryos developed to the blastocyst stage.

We have demonstrated that incubation of bovine cloned embryos under 2% CO_2 with negative air pressure and possibly a reduced O_2 concentration after aspiration of air has a beneficial effect on the development of early-stage cloning embryos. These results suggest that the whole process of bovine cloning embryos in the presence in CR 1aa can be carried out effectively in a portable incubator. In addition, these culture condition may play a key role in subsequent normal development of cloning embryos in vitro.